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345. EXPERIMENTS ON MILKING TECHNIQUE

1. EFFECT OF WASHING THE UDDER WITH HOT WATER
2. EFFECT OF REDUCING MILKING TIME

BY F. H. DODD AND A. S. FOOT
National Institute for Research in Dairying, University of Reading

(With 9 Figures)

REVIEW

The good milker, when working by hand or operating a milking machine, has always milked quietly and as quickly as possible to encourage complete evacuation of the udder. He has realized that efficient milking depended upon the co-operation of the cow and the milker, and that complete 'let-down' of milk depended on the cow's environment and the manner in which the milker operated. Apart from the fairly obvious danger of causing udder injury by leaving the teat cups on the cow too long, the precise value of a rapid milking technique, however, was not realized until evidence of this had been provided by physiologists in quite recent years. In submitting this evidence they have also pointed to certain other necessary factors in efficient milking.

That extracts of the pituitary have a stimulating effect on the mammary gland has been known for many years. In his study of the effect of injection of pituitrin and of nursing by their own offspring in lactating goats and bitches, Gaines (1) concluded as long ago as 1915 that 'milk secretion, in the sense of the formation of the milk constituents, is one thing; the ejection of the milk from the gland after it is formed is quite another thing'. He was able to demonstrate that the sucking of one gland of a goat by its kid caused a copious flow of milk from the other (cannulated) gland about 50 or 60 sec. after commencement of sucking. Only in very recent years has the significance of these findings to the dairy farmer been generally appreciated.

Turner & Slaughter (2) also suggested that pituitrin is not a galactogogue but 'rather acts on the mechanism normally effective during the milking process'. Later Ely & Petersen (3) put forward the theory that 'the delicate balance between the product of the suprarenal medulla, adrenalin, and the oxytocic principle of the posterior lobe of the pituitary body, in the blood of the cow at the time of the milking act, seems to be responsible for the rate of ejection of milk'. Miller & Petersen (4) and Knodt & Petersen (5) have published evidence in support of this theory.

The Minnesota workers and others have drawn attention to the importance of preparing the cow for milking in such a way that the release of oxytocin is as positive as possible and carefully timed so that the let-down of milk coincides with the beginning of milking. Petersen (6), Dahlberg (7), Hopson (8), Fountaine (9) and others in advising on this matter have advocated the use of hot water at say 120–130° F. for washing the udder, and

although there appears to have been no extensive investigation as to the effect of hot-water washing on subsequent milking, this practice has become fairly widespread in the United States and is becoming common in Britain.

Since the effect of oxytocin on the udder is transitory, the need to milk quickly has become clear, and a good deal of attention has been given to the rate of milking. Most of this work has been carried out on machine-milked cows, and the usual method has been to suspend the milking machine unit on a spring balance and then take balance readings at 15, 20, 30 or 60 sec. intervals throughout the milking of each cow. This method has been used by Matthews *et al.* (10), Foot (11), Elting & LaMaster (12). Later an extensive study has been carried out by Matthews *et al.* (13) in which a machine specially constructed to collect the milk from separate quarters into individual containers has been used. More recently, Whittleston (14) has devised electrical apparatus for measuring the rate of flow from the udder. While studies by these workers have provided useful basic data on the rate of flow of milk from the udder under normal circumstances, there is so far not much evidence as to the effect on rate of flow at various stages of milking of methods of preparing for milking or of variations in machine-operation technique, although Smith & Petersen (15) have provided data on the effect of varying the negative pressure and type of pulsation on the rate of flow, and more extensive investigations of this type are being carried out by Whittleston (16) in New Zealand.

There is a good deal of evidence that prolongation of the milking act reduces yield at any one milking. The classic method of demonstrating this is by milking individual quarters in rotation. Babcock (17), Emery (18), Bitting (19), Beach (20) and Crowther (21) have shown that there is commonly a progressive decrease in the amount of milk and fat drawn from successively milked quarters, although the quarter milked second sometimes yields more than the first. Babcock and Crowther also showed that slow milking *per se* reduced production at a milking. Conversely, Dahlberg (22) has reported long-term studies in which a gradual reduction in milking time from about 10 min. to 4 or 5 min. per cow increased annual production.

There is therefore a wide field for investigation into factors influencing the rate of milk flow from the udder in practice. Apart from fundamental considerations, the milking-machine operator is urgently in need of reliable data on such matters as the influence of common environmental factors on the rate of flow, the scope for training cows to a quicker routine, the effect on yield of reducing milking time at the expense of leaving small quantities of milk in the udder. Apart from considerations of yield and economy in milking time there is also the very important matter of the effect of milking technique on mastitis. One aspect of this, that is, the danger of injury by the milking machine when left on the cow too long, has been emphasized by Petersen (23), but on such matters as the effect on mastitis of hand stripping after the machine, somewhat contradictory evidence is available. For example, Woodward *et al.* (24) claim no ill effect when no hand stripping was carried out, while Schalm & Mead (25) reported an increase in clinical cases when cows with subclinical mastitis were not hand stripped after the machine.

Experimental

Two experiments have been completed. The first was designed to find out the effect of two different udder-washing routines, and the second the effect of a gradual reduction in milking time on the yield and quality of milk and on the speed of milking.

Experiment I

Eight Shorthorn cows and one Guernsey, all in declining lactation and yielding at the beginning of the experiment from 22 to 40 lb. milk daily, were used. The animals were housed, managed and fed under normal good conditions. Milking was, however, carried out in seclusion away from danger of extraneous disturbance and at precisely the same time each day—6.45 a.m. and 3.45 p.m., while as little change as possible took place in operators. Stripping was carried out with the teat cups, hand stripping only being resorted to for one cow for a brief period (see below).

The experiment was divided into three periods—a preliminary control period of 2 weeks, an experimental period of 5 weeks and a final control period of 2 weeks.

During the control periods the cows were prepared for milking by the method normally practised at the Institute, that is, the hindquarters, tail and hindfeet were hosed down with cold water, a curry comb or brush being used to move any dung not flushed off; the udders were next washed with a cloth dipped in clean cold water and subsequently dried with a dry cloth and finally the foremilk was drawn off in a strip cup. During the control period each of these operations was carried out in turn throughout the whole group of cows, and after the foremilk had been drawn off along the line a period of 10–15 min. normally elapsed before the first cow was milked. Thus the period between preparation and actual milking varied from about 15 min. for the first cow milked to about 1 hr. for the last cow milked.

During the experimental period the cows were tied up and given their concentrates and were then not touched until each was ready for washing immediately before putting on the teat cups. The animals were not hosed before milking. Any dung on the cow that was likely to come in contact with the operator or the machine was quickly removed with a brush, cloth and water and the whole udder was then completely washed with a cloth thoroughly soaked in clean water at 115° F. (or 120° F.—see below). The udder was then dried with the same cloth and the foremilk removed with a strip cup. These preparations were timed so that the teat cups were put on the cow within about a minute of their completion in all cows. In changing from one routine to another the water temperature was changed in stages over a period of 3 days. At no stage in this experiment was an effort made to train the animals to a quicker routine by removing the teat cups before milking had substantially ceased.

Results

The mean daily milk yield for each week for the group, is set out in Table 1. Fig. 1 shows the mean yield of the group each day throughout the experiment. The general impression obtained from Table 1 and Fig. 1 is that the decline in yield continued fairly consistently.

Three points are worth noting. The first is the apparent greater consistency in day-to-day yield during the experimental period compared with the control periods. The second is the decrease in rate of decline during the second half of the experimental period, and the third is the slight drop in yield when the change from the experimental period to the final control period took place. The two latter points are more clearly seen if the weekly decline in yield of the whole group is studied. The figures obtained by subtracting the

Table 1. *Summary of mean daily milk yield, fat percentage, total fat and solids-not-fat percentage*

Week	Mean yield (lb./day)	Mean fat %	Mean total fat (lb./day)	Mean S.N.F. %
		Control		
1	28·45	3·74	1·05	8·92
2	27·05	4·02	1·06	8·90
		Experimental		
3	25·83	4·07	1·04	8·99
4	24·65	4·06	0·99	8·97
5	24·00	3·97	0·94	9·04
6	23·72	4·03	0·94	9·07
7	23·76	4·15	0·98	8·99
		Control		
8	22·21	4·18	0·92	9·00
9	21·80	4·09	0·87	8·95

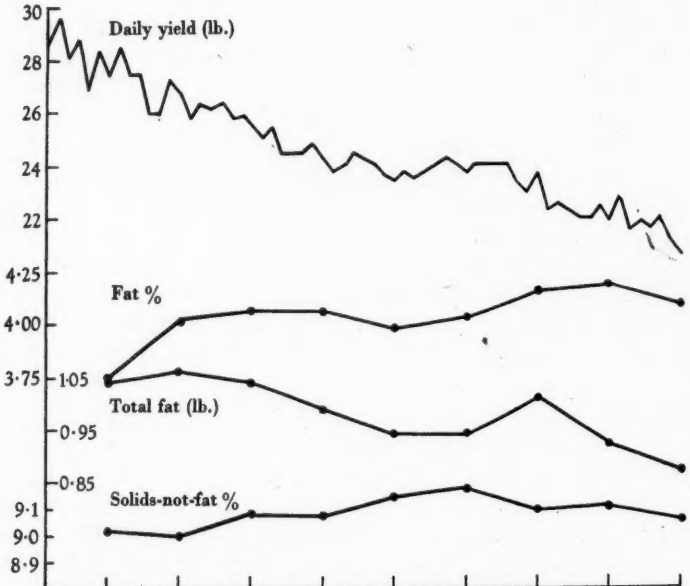

Fig. 1. Mean daily yield. Mean daily yield of fat. Mean solids-not-fat percentage and mean fat percentage. For cows in Exp. I.

daily yield during the second week from that of the first, and the third from the second, etc., are as follows:

Control	1st–2nd	1·40 lb.
Experimental	2nd–3rd	1·22 lb.
	3rd–4th	1·18 lb.
	4th–5th	0·65 lb.
	5th–6th	0·28 lb.
	6th–7th	0·06 lb. (gain)
Control	7th–8th	1·55 lb.
	8th–9th	0·41 lb.

These certainly suggest that the hot-water washing gradually reduced the rate of decline in yield. The effect, however, is very small, and in view of the absence of a control group or of comparative 'normal' lactation curves, it is unwise to claim more than that the hot water had no detrimental effect on yield.

Chemical composition. The fat percentage, solids-not-fat percentage and fat yield are given in Table 1 and Fig. 1. The mean fat percentage shows a general rise throughout the experiment—a result that would be expected in view of the advance in lactation of the cows. The fat-yield data reflect the changes in milk-yield data recorded above. The upward trend in solids-not-fat percentage during the experimental period is an interesting feature, since this could not be explained on the basis of advancing lactation, and in any case there appeared to be a drop in the final control period.

Speed of milking. From the data collected on the speed of milking of the individual cows the mean time that the teat cups were on the cows at the a.m. milking was as follows:

	Min.	Sec.
Initial control period	7	40
Experimental period	7	15
Final control period	7	7

These figures, however, depend on the operator's judgement as to the end of milking and on the time taken to strip with teat cups. Hence a better comparison of the relative speed of milking under the two washing routines is provided by graphs of the rate of milk extraction at intervals after the teat cups were put on the cow. In order to give these data in a concise pictorial form composite curves have been constructed from data collected at morning milkings on about five occasions during the experimental period and on five occasions during the control periods. These are set out in Fig. 2. In these graphs the solid line shows the mean rate of milk flow from the time the teat cups were put on the cow until near the end of milking during the experimental period when the washing was by hot water immediately before milking. The broken line gives a similar graph for the mean data collected during the two control periods. It is not possible to complete these composite curves to the point where machine stripping was commenced owing to variation in the duration of milking from day to day.

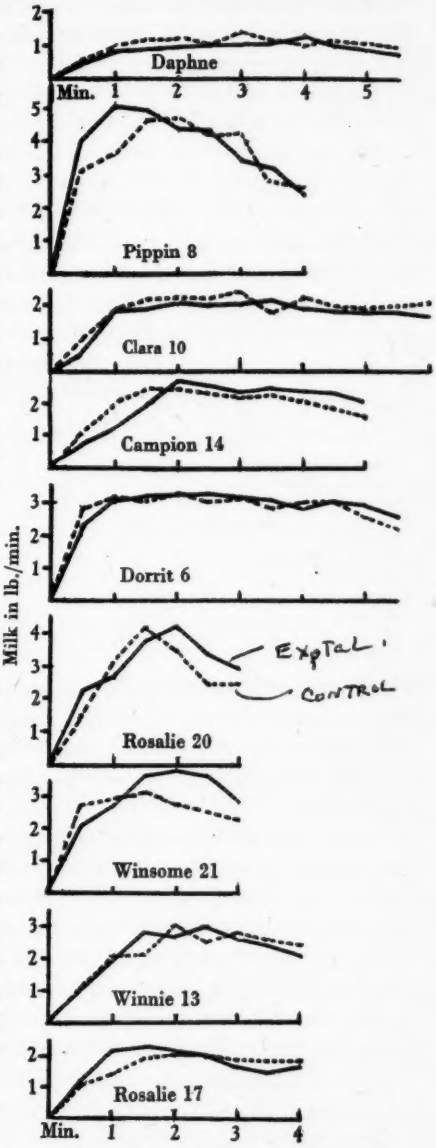

Fig. 2. Rate of flow—nine cows—Exp. I.

In general, these curves indicate that there was very little difference in the rate of milk flow following the two different washing routines. There is some slight evidence of an earlier peak in the flow with Pippin 8 when washed with hot water, but the only cow showing a pronounced difference in the profile of the two graphs is Winsome 21, who not only showed a slower maximum rate of flow with the cold-water method but also retained a substantial proportion of her milk as strippings when under this routine.

The results of the Whiteside test for udder abnormality shown in Table 2 indicate that the milk was normal to this test throughout, apart from isolated positives. These were not persistent except in the case of Dorrit 6's right hind, which probably gave this result because she gave little milk in that quarter. The positives in the case of Rosalie 17 and Daphne were due to mastitis. Those in the case of Winsome 21 and Clara 10 may have been associated with chapped teat orifices, since apart from micrococci in one case no bacteria were found associated with their high Whiteside results.

Table 2. *Whiteside tests, Experiment I*

	Initial control period								Experimental period							
Date ...	22. x. 45				2. xi. 45				23. xi. 45				14. xii. 45			
Cow	L.H.	R.H.	L.F.	R.F.	L.H.	R.H.	L.F.	R.F.	L.H.	R.H.	L.F.	R.F.	L.H.	R.H.	L.F.	R.F.
Clara 10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4+	3+
Campion 14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Daphne	±	2+	±	±	.	.	.	.	.	.	.	.	.	.	.	.
Dorrit 6	.	±	.	.	.	±	.	.	.	1+	.	.	.	2+	.	±
Pippin 8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4+	.
Rosalie 17	.	.	.	.	.	.	.	.	.	.	4+	.	.	±	1+	.
Rosalie 20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Winnie 13	4+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Winsome 21	.	±	.	.	.	.	.	.	.	.	.	.	.	.	2+	2+

L.H. left hindquarter; R.F. right forequarter.

All cows were tested bacteriologically for mastitis 8 days before going on experiment and all were negative. They were again all negative on the 23rd day after commencing the experiment, and all except one (Clara 10, see below) were negative at the end of the experimental period. Between these routine tests, however, the following abnormalities were noted:

Daphne: Clots in the right hindquarter 12 days before commencement of experiment. Negative to bacteriological test but positive to Whiteside test. A penicillin infusion was made on the following day, but the clinical condition and positive Whiteside test persisted for 2 weeks. Five subsequent bacteriological tests were negative.

Rosalie 17: Clots in right hindquarter 29 days after commencement of experiment. Bacteriological test showed staphylococcal infection of the right hindquarter. The quarter apparently returned to normal following four penicillin infusions. Five bacteriological samples during the following 6 weeks were negative. Slight clots in the left hindquarter reappeared 23 days later, i.e. 5 days before the end of the final control period, but these disappeared after 3 days without treatment, and at a subsequent examination the quarter was negative.

Clara 10: On two occasions at the end of the experimental period, *Streptococcus uberis* was recovered in rather large numbers from the left hindquarter. She showed no symptoms of clinical mastitis, the Whiteside test was negative and was negative to the bacteriological test 16 days after the end of the experiment.

These abnormalities are similar to those which have been experienced in the herd where critical observation of the health of the udder has been made under a normal milking regime, and there is no reason to associate them with the hot-water treatment. There was no evidence of injury to the skin of the udder or teats. During the first 17 days of the experimental period the washing water used was at 120° F. and there was slight evidence of greater udder sensitivity particularly in the case of Campion 14 who began to kick occasionally. For this reason the temperature of the water was reduced to 115° F., and no further trouble of this kind developed. Throughout the whole period the teats were in good condition and sores, etc., were rare.

Experiment II

Six Ayrshire cows kept on a commercial farm in the vicinity of the Institute were chosen so that there were three pairs matched for age, stage of lactation, yield, and ease and speed of milking. The cow to receive the experimental treatment in each pair was chosen by lot and the relevant data for the six cows are given in Table 3.

Table 3. *Details of cows used in Experiment II*

Name of cow	Pair	No. of calves	Date calving	Approx. yield at beginning lb. daily	Type of milker
Experimental group:					
Kate	I	1	19. xi. 45	30	Fast
Wincey	II	2	6. x. 45	31	Slow
Primula	III	4	21. xi. 45	33	Fairly slow
Control group:					
Buttercup	I	1	14. xi. 45	25	Fast
Sunflower	II	3	30. x. 45	37	Fairly slow
Etonia	III	2	12. xii. 45	35	Slow

The cows were of good type with well-shaped udders and had always been milked by machine.

A bucket-type milker was used and was operated at 50 pulsations per minute and negative pressure of 13 in. of mercury. Milking was carried out twice daily commencing at 6.30 a.m. and 4.00 p.m. The six cows on experiment were stripped with the machine. The same operator milked the cows throughout, apart from a very few occasions when another experienced operator did the work. The handling of the machine and the stripping was efficient.

Apart from the three experimental cows, during the last 3 weeks of the experimental period all udders were washed with cold water at the same time as the other cows in the herd before the milking machine motor was started. The udders of the three experimental cows were washed with water at 115° F. immediately before putting on the teat cups at all milkings during the last 3 weeks of the experimental period with the object of finding out if this change of washing technique had an effect when introduced on cows already under rigorous training to milk more quickly.

Hypochlorites to provide about 800 p.p.m. chlorine were added to the udder washing water and to the water used for dipping the teat cups between each cow.

The cows were maintained under good herd management and were fed with the rest of the herd. During the 11 weeks of the investigation the normal change from winter feeding to summer grazing was taking place. Owing to the fact that the spring growth of

grass began early but failed to reach a pronounced flush during the experiment, the gradual increase in nutrients from grazing approximately compensated for advancing lactation throughout the period, consequently the yield of the cows generally remained fairly steady.

The animals were used to receiving their concentrates as they were milked, and this practice was continued throughout the experiment.

Timing of milking and stripping of all cows at all milkings was carried out by stop-watch. The time taken to milk and to strip each of the control cows was left entirely to the judgement of the operator, who put the teat cups on and took them off when he considered milking was complete, subject to some slight delay at times when he was occupied with another unit. The experimental cows were treated in a similar way during the initial and final control period, but during the experimental period they were restricted to a precise time schedule. The stripping action was commenced and the teat cups taken off at fixed intervals after they were put on. The time schedule was based on the normal morning milking time and normal evening milking time as assessed for each cow during the initial control week. The experiment lasted 11 weeks from 8 March to 23 May 1946, there being 1 week initial control period, 8 weeks experimental period and 2 weeks final control.

The schedule of the experimental period given as approximate percentages of normal milking time was fixed as follows:

Weeks ...	1st	2nd	3rd	4th	5th	6th*	7th*	8th*
Kate	100	90	80	70	60	60	60	60
Primula	100	90	80	70	70	70	70	60
Wincey	100	90	80	70	70	70	70	60

* Udders washed with hot water during 6th, 7th and 8th weeks.

Stripping times based on the normal for the initial control week were fixed for a.m. and p.m. milking of each experimental cow, and these were not changed throughout. (Although the term 'stripping time' is used throughout this experiment, certain of the animals were in fact in full flow during 'stripping' in the later weeks—see below.)

The milk of each cow was weighed at each milking; samples were taken at a.m. and p.m. milking twice weekly for fat test and S.N.F. estimation.

Bacteriological tests for mastitis were made on individual quarters of all animals on two occasions before the start of the experiment and on two occasions at the end of the experiment. Weekly tests were made in the case of Primula (see below). Whiteside tests were carried out on individual quarters of all cows at weekly intervals.

By taking readings at 15 sec. intervals from a spring balance on which the milking unit was suspended, data concerning the rate of milking of all cows, a.m. and p.m., were obtained at weekly intervals.

Results

Yield. The mean daily milk yield of the experimental and control groups is given in the first column of Table 4 and in Fig. 3. The yields of the two groups were originally very similar, and although the yield of the control group tended to exceed the yield of the experimental group, especially in the second half of the experiment, the difference is small, and a simple statistical test of the difference in regression of the two graphs showed this to be not significant.

As the experiment progressed it became apparent that the time available for milking the experimental cows was insufficient to allow all the milk in the udder cistern to be drawn; the amount of milk remaining in the udder cistern in the case of Primula and Wincey was apparently frequently considerable, while in the case of Kate the amount varied but was probably usually not great. It was noticeable that most milk was usually left in the hindquarters, which is in agreement with the findings of Smith & Petersen (15). At one milking in the 6th week the operator was allowed to go over the time limit and clear the udder cistern with the object of finding how much milk was being left in the cistern. On this particular occasion, however, only Wincey yielded appreciably more than she had been giving on the restricted time table, and she gave about 5–6 lb. extra. At the end of the 9th week, when the final control period started and the udders were milked out, another estimate of the milk previously left in the udder became available. On this occasion, following a week of milking to 60% of original normal milking time, extra milk was obtained to the following extent: Wincey about 6 lb., Primula about 12 lb., Kate about 4 lb. Having disposed of this surplus milk the cows then returned to about

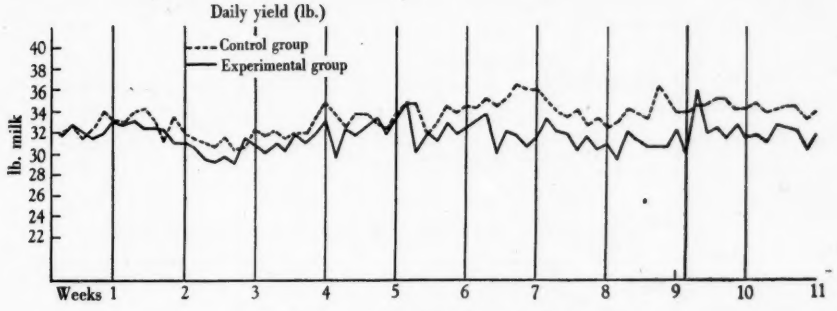

Fig. 3. Mean daily milk yield in Exp. II.

the same daily yield. It is interesting to note that in spite of the fact that these cows were usually not completely milked out for 5 or 6 weeks, the depression in yield was not significant.

Milk quality. The mean fat percentage and mean S.N.F. percentage of the milk of the two groups are given in columns 2 and 3 of Table 4 and in Fig. 4. The S.N.F. level in the two groups remained remarkably similar throughout. The trend of the two graphs is probably associated with changes in the nutritional status of all animals due to improved grazing conditions.

There is evidence that the experimental treatment lowered the fat content of the milk—a result which normally would be expected in view of the incomplete milking of the experimental animals, although in view of the lack of substantial effect on output the lowering in fat percentage might not have been expected to persist.

Speed of milking. The mean time of milking and machine stripping for the two groups is shown in columns 4 and 5 of Table 4, and the mean weight of milk drawn per minute of milking (including stripping time) each day is shown in Fig. 5. With restriction in time there was, as would be expected, a substantial increase in the amount of milk drawn per minute of milking in the case of the experimental group. In the case of the control group there was also an upward trend as the experiment progressed. It is a little difficult

Table 4

Week	Mean daily yield (lb./day)		Mean fat %		Mean S.N.F. %		*Mean daily milking time (a.m. and p.m.) (min.)		†Mean daily stripping time (a.m. and p.m.) (min.)		‡Mean daily milking rate (lb./min.)	
	Exp. group	Control group	Exp. group	Control group	Exp. group	Control group	Exp. group	Control group	Exp. group	Control group	Exp. group	Control group
1	32·18	32·68	3·93	4·07	8·84	8·88	13·69	13·21	2·67	3·13	2·43	2·69
2	32·02	33·69	3·68	4·28	8·71	8·82	13·75	13·54	2·33	2·72	2·45	2·54
3	30·05	31·12	3·69	4·49	8·72	8·75	12·42	12·97	2·33	2·65	2·59	2·53
4	31·23	32·58	3·89	4·16	8·69	8·66	11·00	13·69	2·33	3·06	3·05	2·59
5	31·94	33·44	3·89	4·15	8·73	8·78	9·66	13·36	2·33	2·06	3·48	2·75
6	32·31	33·85	3·70	4·07	8·66	8·75	9·33	12·89	2·33	1·75	3·71	2·85
7	32·02	35·50	3·46	3·76	8·78	8·85	9·33	13·27	2·33	2·19	3·88	2·91
8	31·53	33·71	3·55	3·96	8·77	8·86	9·33	12·88	2·33	2·67	3·74	2·93
9	30·42	34·07	3·74	4·02	8·74	8·84	8·25	12·32	2·33	2·15	4·02	3·19
10	32·53	34·67	3·44	3·51	9·03	9·07	12·50	12·25	2·33	1·66	2·86	3·20
11	31·86	34·19	3·43	3·69	8·96	8·95	—	—	—	—	—	—

* Milking time: The duration of the milking operation from the time the teat cups are put on until their removal—a.m. milking plus p.m. milking.

† Stripping time: The duration of the period of machine stripping—a.m. milking plus p.m. milking.

‡ Milking rate: Milking time/yield.

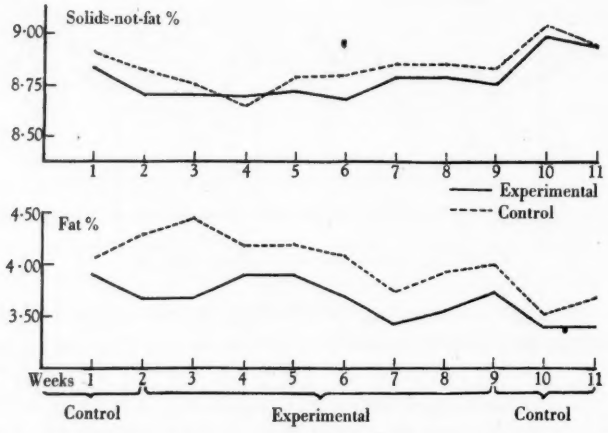

Fig. 4. Mean weekly fat percentage and solids-not-fat percentage in Exp. II.

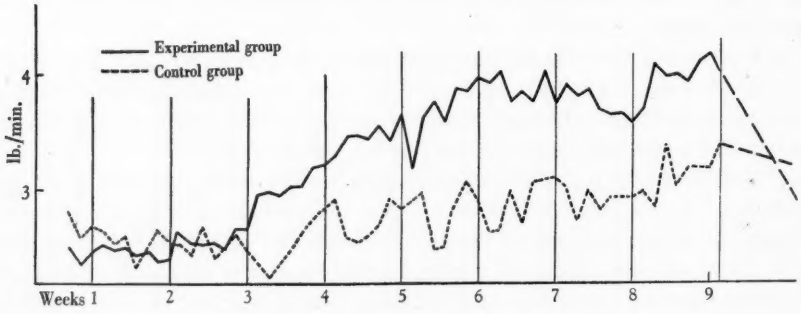

Fig. 5. Mean daily rate of milking in lb./min.

to account for this, as the cows were giving about the same yield throughout and were milked by the same operator. The constant presence of a stop-watch may have caused the operator unconsciously to develop a slightly quicker technique with the control animals.

The rate of flow throughout the actual milking operation on individual cows is shown graphically for a.m. milkings in Figs. 6 and 7. The similarity of the graphs for the same cow when taken at weekly intervals is clearly shown in the case of the control cows. At the same time, the variation from cow to cow and the peculiarities of the shape of the graphs for some cows is illustrated, e.g. the graph for Buttercup is typical for a quick milker, while that for Sunflower is that for a rather slow milker. Etonia was not only slow but apparently habitually delayed the release of her milk after the teat cups were put on.

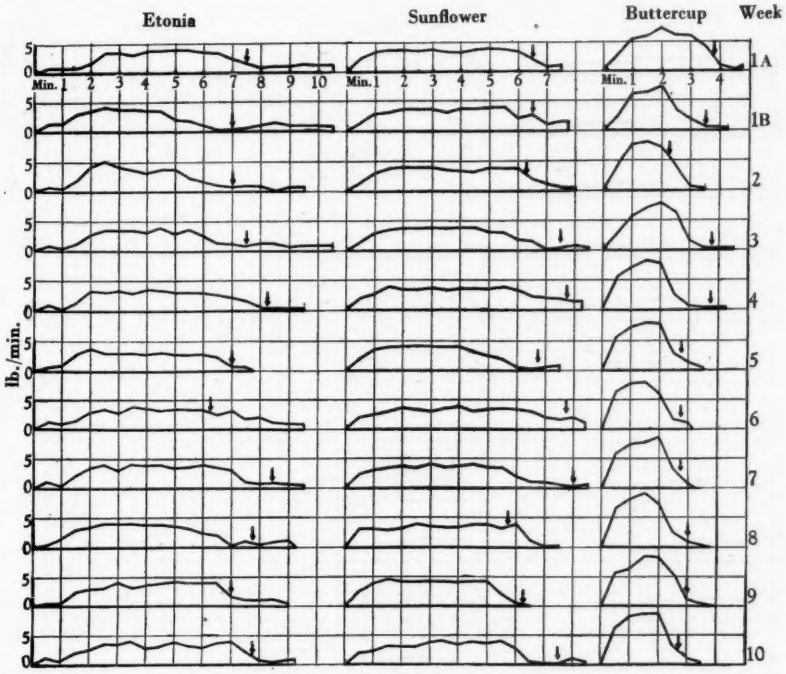

Fig. 6. Rate of flow—three control cows—Exp. II.
Arrow = point at which teat cups were manipulated.

The graphs for the experimental cows (see Fig. 7) illustrate similar points, and also show that as the experiment progressed stripping was commenced and the teat cups removed often while the milk was at full flow. In general, there seemed little inclination on the part of Wincey and Primula to accommodate their flow to the gradually restricted milking time. These cows might have been expected to develop a graph more similar to Kate's or Buttercup's, but in fact their peak flow only increased a little and the profile (apart from amputation of the tail of the graph) remained much as before. Kate, who was initially a fairly fast milker, showed a little more resilience and may have developed an appreciably faster peak flow to suit the changed circumstances. It is, however, hard to draw a conclusion in view of the fact that one of the control cows, Buttercup, also showed a tendency to increase her peak rate of flow.

During the last three experimental weeks hot-water washing of the udders was introduced and there is a possibility that this had some slight effect on the rate of flow, there being a tendency to reach the peak flow a little sooner after putting on the teat cups. This is illustrated more clearly in Fig. 8, which shows for each experimental cow (A a graph of the accumulation of milk in the unit with advancing time after putting on the teat cups during an a.m. milking in the 1st week of the initial control period, (B) similar graphs for the last experimental week; and (C) similar graphs for the 1st week of the final control period. The slight displacement of graph (B) away from (A) and (C) in all three experimental cows may be partly due to training or to hot-water washing or to both of these factors. However, in Fig. 9, where comparable graphs are given for the control

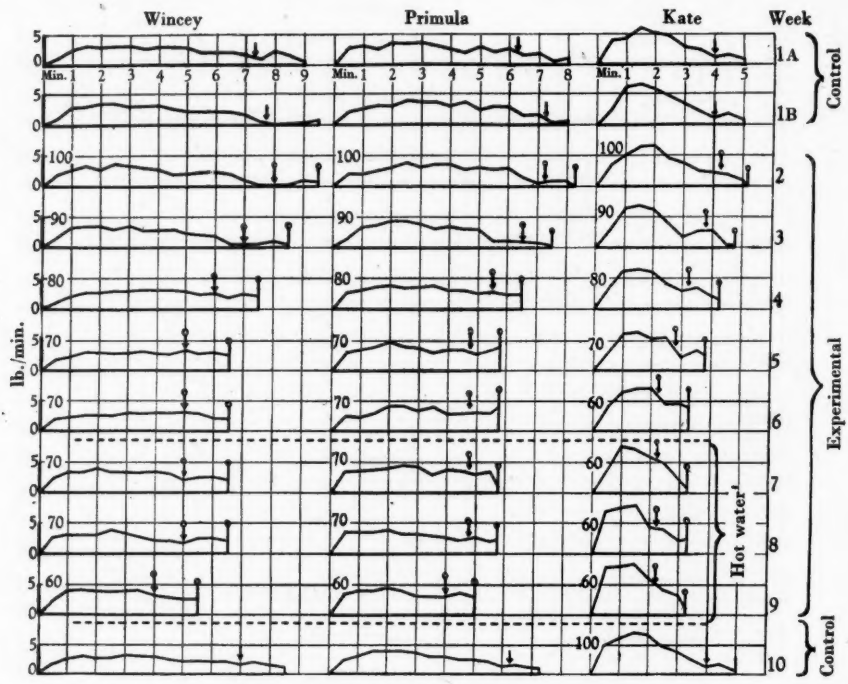

Fig. 7. Rate of flow—three experimental cows—Exp. II.
Arrow = point at which teat cups were manipulated.

cows, the graph (B) in two cows is also very slightly steeper than graphs (A) and (C), and it is again difficult to draw a final conclusion from the data. An effect is perhaps more clearly illustrated by the data given in Table 5, where the amount of milk drawn in the first 2 min. of milking (a.m. and p.m.), on each day, on which rate of flow was studied, is set out. The increase in yield in 2 min. of the experimental cows in the 8th, 9th and 10th week as compared with the control cows is fairly clear-cut. It is, incidentally, worth noting that whereas five of the cows usually gave more milk in the first 2 min. at the morning milking compared with the afternoon milking, the cow Etonia, who had the delayed release, usually gave more milk in the first 2 min. at the afternoon milking. It is difficult to account for this peculiarity.

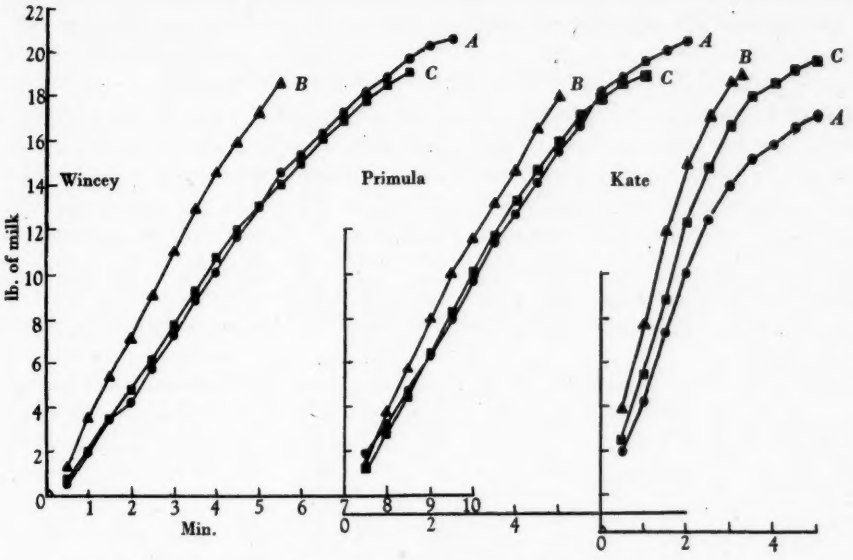

Fig. 8. Accumulation of milk in the milking machine bucket during milking—three experimental cows—Exp. II.

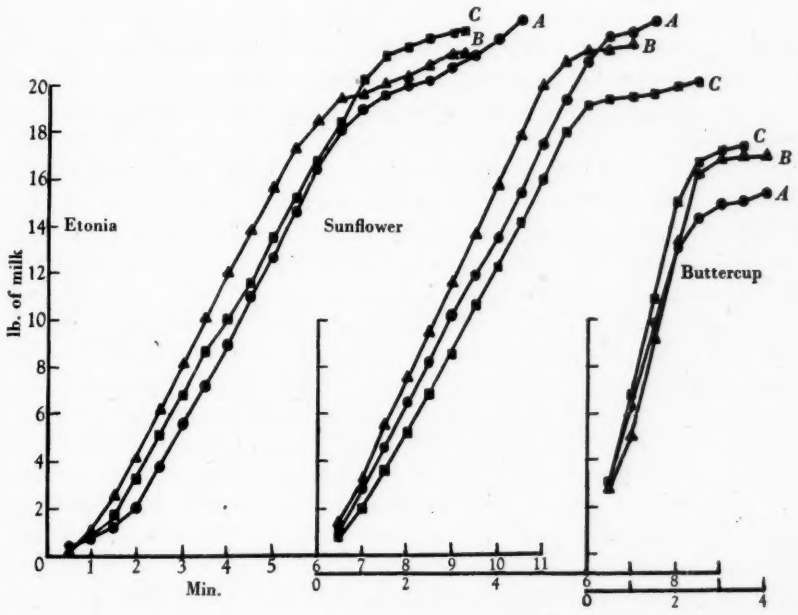

Fig. 9. Accumulation of milk in the milking machine bucket during milking—three control cows—Exp. II.

Notes on the efficiency of milking as judged by palpation of the udders after the teat cups were removed indicated a fairly big day-to-day variation in the amount of milk left in the udders of the experimental animals.

Table 5. *Milk drawn (lb.) in 2 min.—Experiment II*

Week ...	1		2		3		4		5		6	
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
Primula	6·6	5·6	5·5	5·9	5·3	5·8	7·0	4·4	6·5	6·4	7·2	6·5
Wincey	4·9	3·3	5·3	3·9	5·6	4·1	6·1	3·9	4·3	3·4	4·8	4·3
Kate	10·0	9·5	10·6	10·0	11·2	9·8	11·3	9·7	10·8	11·6	10·4	11·2
Mean	7·16	6·13	7·13	6·6	7·36	6·56	8·13	6·0	7·2	6·8	7·46	7·33
Sunflower	6·5	5·1	5·8	5·4	6·5	6·0	6·0	4·8	6·0	5·9	7·0	5·1
Buttercup (1½ min.)	9·9	8·3	10·6	8·8	10·0	8·5	10·3	9·0	10·5	10·9	10·6	8·4
Etonia	2·1	3·5	5·1	5·0	3·5	4·0	2·9	4·8	3·6	4·0	4·2	3·3
Mean	6·16	5·63	7·16	6·40	6·66	6·00	6·40	6·20	6·70	6·93	7·26	5·6

Week ...	7		8		9		10		11	
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
Primula	5·6	6·7	7·5	6·6	7·2	6·6	8·0	7·0	6·3	5·3
Wincey	4·7	4·7	6·5	6·2	5·7	6·4	7·1	6·5	4·8	4·8
Kate	13·0	11·7	13·4	12·6	13·5	11·2	16·2	11·8	11·3	9·9
Mean	7·76	7·70	9·13	8·46	8·8	8·06	10·1	8·43	7·46	6·66
Sunflower	5·5	5·8	6·2	6·1	6·0	6·0	7·5	6·2	5·2	5·6
Buttercup (1½ min.)	12·2	9·0	10·5	10·8	11·8	9·4	9·2	10·7	10·9	11·3
Etonia	3·2	3·5	3·6	3·3	3·2	5·2	3·6	4·2	3·3	3·8
Mean	7·30	6·10	6·76	6·73	7·0	6·86	6·66	7·03	6·46	6·90

	Mean a.m.	Mean p.m.
Control	6·77	6·40
Experimental	7·97	7·21

Table 6. *Results of Whiteside test, Experiment II*

	7. iii. 46				9. iii. 46				20. iii. 46				27. iii. 46			
	R.F.	R.H.	L.F.	L.H.	R.F.	R.H.	L.F.	L.H.	R.F.	R.H.	L.F.	L.H.	R.F.	R.H.	L.F.	L.H.
Experimental group:																
Wincey	3+	±	2+	±	±	.	±	.	1+	±	2+	2+	1+	.	1+	.
Kate	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Primula	.	.	.	.	3+	1+	.	.	2+	.	.	.	±	.	.	.
Control group:																
Buttercup	.	.	.	.	.	.	2+	.	.	.	.	.	.	.	.	.
Etonia	.	.	.	1+	.	.	.	.	.	.	.	.	.	.	.	.
Sunflower	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	4. iv. 46				10 iv. 46				17. iv. 46				24. iv. 46			
Experimental group:																
Wincey	1+	.	±	.	1+	.	2+	±	1+	.	1+	.	2+	±	1+	.
Kate	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Primula	.	.	.	.	.	.	.	.	2+	.	1+	±	3+	.	±	±
Control group:																
Buttercup	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Etonia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Sunflower	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	1. v. 46				9. v. 46				10. v. 46			
Experimental group:												
Wincey	1+	.	1+	.	1+	.	±	.	1+	±	2+	.
Kate	.	.	.	.	.	.	.	.	.	.	.	.
Primula	2+	.	±	1+	1+	.	.	.	2+	±	.	.
Control group:												
Buttercup	.	.	.	.	.	.	.	.	.	.	.	.
Etonia	.	.	.	.	1+	.	.	1+	.	.	.	.
Sunflower	.	.	.	.	±	±	1+	±	.	.	.	.

Udder health. Five of the cows used in this study had, prior to the experiment, never shown evidence of clinical mastitis. Primula had had clinical mastitis in her right forequarter in a previous lactation. A bacteriological test of milk from individual quarters on two occasions before the commencement of the experiment showed the five to be free from subclinical mastitis. These animals were also free from subclinical mastitis when examined bacteriologically on two occasions near the end of the experiment. Primula showed a staphylococcal infection in the right fore- and left hindquarters at the pre-experiment test, and subsequent weekly examination of the milk from individual quarters of this cow showed the infection to persist. Thirty-nine days after the beginning of the experiment the mastitis became clinical in that clots were found in the foremilk from the right forequarter. 100,000 units of penicillin were injected into the quarter on two consecutive days, and although the clots did not entirely clear till 5 days later the infection subsided. The quarter subsequently continued to carry a subclinical infection, but has not so far been reported clinical.

The results of the Whiteside test for abnormal milk are given in Table 6. By this test, Wincey's milk was slightly abnormal throughout, but showed no tendency to change with experimental treatment. Primula's results reflected the development of clinical mastitis.

Discussion

Possibly the most important aspect of the results reported above is the absence of any spectacular change in the rate of flow of milk from the udder of any particular cow in spite of rather drastic changes in preparation and milking technique. The similarity of the flow curves for the same cow on different days and under different routines is notable. It must, however, be borne in mind that these experiments are short-term, and quite different results may be obtained in long-term experiments, especially where differential techniques are introduced on the freshly calved heifer. Assuming a milking machine operating consistently, the rate of flow from any udder for a given quantity of milk will depend on (*a*) the let-down mechanism and (*b*) the anatomical structure of the ducts, udder cistern, teat sinus and teat orifice. A substantial change in the latter due merely to changes in udder-washing technique would hardly be expected to be apparent in a period of a few weeks, and the flow curves certainly suggest that it is mainly these physical factors that limit the maximum rate of flow. Delayed let-down appeared to prolong milking only in exceptional cases in our experiments, although in the first experiment the more rapid let-down in the case of Rosalie 20 and more effective let-down in the case of Winsome 21, where hot-water massage immediately before milking was practised, are notable. Also, in the second experiment Etonia's natural delay in let-down compared with other cows on the experiment is of interest. It is also worth noting that whereas the cows in Exp. 1 failed to run their milk after washing during the initial control period, most of them ran a substantial amount after washing during the first few days of the final control period. In an experiment comparing the yield of cows when washed 20 min. before milking with the yield of the same cows when washed immediately before milking, Miller & Petersen(4) found a 5% greater milk yield in favour of washing immediately before milking. The experimental treatment (washing 20 min. before milking) was only carried out over a 1-week period, and the yield difference may have been due to *change* in washing technique *per se*. It is now commonly accepted that the let-down of

milk is a conditioned reflex, and in our work we found evidence of a brief drop in yield when changing to hot wash immediately before milking and also when changing to cold wash some time before milking. However, although the clear-cut evidence of the advantage of udder washing immediately before milking or of the use of hot water for this purpose is yet to be published, logically and physiologically there is much to be said in favour of these practices.

Our inability to increase the peak flow in spite of a gradual reduction in actual milking time to 60% of previous normal, and in spite of removal of teat cups before milking was complete at each milking for 3 or 4 weeks, is not necessarily contrary to the experience of Dahlberg (7), who gradually decreased average milking time from about 10 min. to 4 or 5 min. per cow without decreasing yield. A good deal of his time economy may have been due to elimination of the common fault of leaving the teat cups on the cow after milk flow has substantially ceased. In some cows he may also have eliminated the last part of the flow period, but our results have shown that this can be done without considerable drop in yield. This absence of decline in yield due to retained milk in our second experiment may be partly due to the fact that the cows were in mid-lactation, and as a result udder pressure sufficient to inhibit secretion may not in any case have occurred till the approach of the succeeding milking. Also, grazing conditions were improving as the experiment progressed, and this may well have helped to maintain yields relative to the control animals. In a period of falling yields retained milk may be more effective in reducing persistency of lactation. It has been suggested (6) 'in the change-over (to quicker milking) little loss of production will occur, most cows not being affected and those falling in production will return to normal in a few days', but even where allowance is made for difference in yield of individual cows it certainly would seem from our results that actual training to a quicker flow is not merely a matter of gradual reduction in milking time over a few weeks to any preconceived time limit. It is possible that long-term training may achieve this object. There is also a great deal that can be said in favour of removing teat cups as soon as flow has ceased, but short of drastic changes, such as increasing the vacuum, a quick change in normal speed of milking appears to be difficult to attain.

The data on udder infections are insufficient to merit conclusions other than indicating that the use of hot water (about 115° F.) for washing udders has no pronounced effect on the incidence of clinical mastitis. Primula's mastitis during a period when milking time was restricted is of little significance by itself and Whiteside's results showed no evidence of abnormal milk in two cows treated similarly.

Summary

1. During the first 2 weeks and last 2 weeks of a 9-week period nine cows, milked by machine, were prepared for milking by hosing and washing the udders with cold water 15 min. to 1 hr. before milking, while in the intervening 5 weeks the udders only were washed with water at 115 to 120° F. immediately before milking.

2. The hot-water routine had no pronounced effect on the yield and quality of the milk from the majority of the cows, although one reacted unfavourably to a change back to the cold water routine and had to be stripped by hand.

3. The gradual restriction, over an interval of 4–6 weeks, in the milking time from 100 to 60% of the previous normal flow period for three cows in mid-lactation had no great influence on the maximum rate of milk flow from the udder, even when hot-water

washing of the udder immediately before milking was introduced late in the experiment. The maximum rate of flow was, however, reached slightly sooner after putting on the teat cups.

4. The restriction in milking time caused only a small reduction in yield as the flow during the latter part of the flow period had in any case been small.

5. The fat content of the milk of the cows whose milking time was restricted fell somewhat while the solids-not-fat content remained steady.

6. Washing the udder with hot water or restricting milking time showed no clear-cut effect on the incidence of mastitis.

Our thanks are due to Dr S. J. Rowland, who carried out the tests for chemical quality of the milk, and to Mr F. K. Neave, who carried out the bacteriological examination for mastitis. We wish also to record our appreciation of the help given by Messrs J. Povey and Sons, of Swallowfield, who not only provided cows for the second experiment but co-operated willingly in the collection of data.

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346. MEASUREMENTS OF THE RATE OF CHANGE OF TEMPERATURE IN HIGH TEMPERATURE-SHORT TIME PASTEURIZATION PLANT

By F. STEGHART, *Tinsley (Industrial Instruments) Ltd., London*

(With 9 Figures)

It has recently been claimed that in modern high temperature-short time pasteurization plant fluctuations in temperature of the order of 1° F./sec. are unusual and probably artefacts, and that an instantaneous drop is certainly fictitious.

It has, nevertheless, been shown that such rapid drops in temperature do in fact occur frequently in high temperature-short time plants of the type investigated. The plant investigated was not of the latest design incorporating devices for speeding up the control by injecting steam directly into the hot-water pipe.

Temperature changes of the order of those in question were first observed by Mattick & Hiscox(1) of the National Institute for Research in Dairying, who carried out tests on pasteurization plant using a small mirror galvanometer with a very short time constant. The maximum rates of change were, however, not observed.

Some two years ago, new equipment, for the operation of the flow-diversion valve, which included a high-speed indicator and a recorder, was devised. The time response of the latter was not better than 1 sec., but observation of the high-speed temperature indicator showed that the rate of change of the temperature sometimes considerably exceeded 1° F./sec. A high-speed recorder was, therefore, included in the circuit, and the observations recorded in the graphs proved the occurrence of the very high rates of change.

It is of great importance to know the maximum rate of change of temperature because it determines not only the type of equipment that is necessary for the operation of the flow-diversion valve but influences the lay-out of the heat exchanger and of the whole pasteurizing plant. If that speed is underestimated, it may happen that milk will pass through underheated. If, on the other hand, the speed is overestimated, the equipment may be designed with unnecessary complications.

The test arrangement attached to a plate pasteurizer is shown in Fig. 1. The thermocouple *B* was placed not immediately at the inlet but about one-third up the holder, so that the fluid under treatment had passed the two grids which help to promote mixing and decrease the rate of temperature change.

A cold junction, an amplifier and a high-speed recorder were connected to thermocouple *A* and a second similar arrangement to thermocouple *B* (Fig. 1). Rapid changes of the temperature of the milk were produced almost at will by making a quick change of the filter or, alternatively, by taking the plant into operation. While rapid changes could be observed from thermocouple *B* it was not possible to observe them from thermocouple *A*, where the fastest rate of change observed never exceeded 1° F./sec. because rapid changes were damped out in the holder.

The first conclusion to be drawn is, therefore, that high-speed diversion equipment must be placed at the inlet to the holder. At the outlet from the holder this equipment is far less valuable.

Since the thermocouple *B* was arranged some way up the holder it is obvious that even the high-speed temperature-measuring equipment used in these tests, which is 10–20 times faster than other similar apparatus, may in special circumstances not be fast enough for research purposes. The equipment used operates within 0·1 sec. It would, however, at present be useless for practical purposes to exceed this speed of response on industrial equipment because the operation of the flow-diversion valve cannot at present be reduced below this figure.

The tests were carried out only in one type of H.T.S.T. pasteurizer, but there is little likelihood that other H.T.S.T. equipment would behave very differently. It seems that the tests carried out in the past were made with equipment of which the rate of response was too slow and observations did not extend over a sufficient period.

The use of multiple couples must be avoided because it is impossible to measure the temperature at one point, and the distribution of couples over any length of pipe or

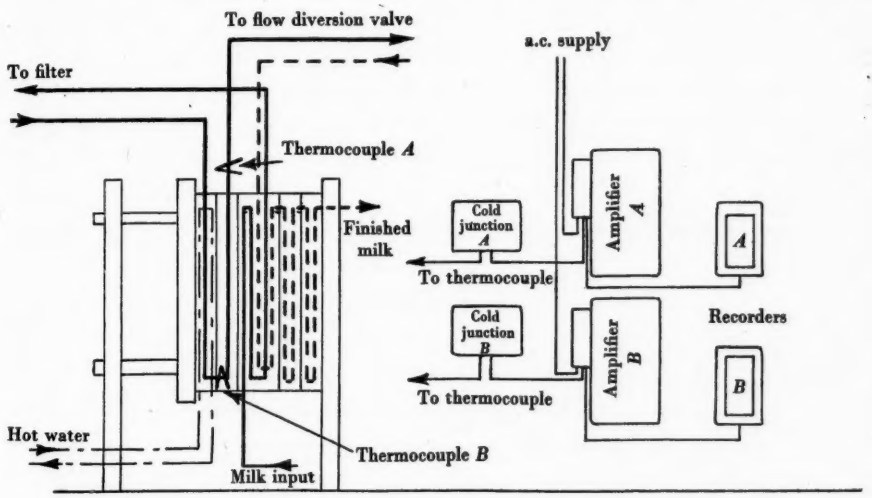

Fig. 1.

across the diameter tends to smooth out the temperature gradient of fast wave fronts. To test the stability of the equipment both equipments were left in operation for some hours before test observations were made. The strips of one recorder moved at a speed of $\frac{1}{2}$ in./sec. and the other with a speed of 1 in./10 sec. During the whole period of about 25 hr. neither one of the records of the two equipments deviated from a straight line except when operations on the plant known to produce deviations were carried out.

The type of record ordinarily obtained is shown in Fig. 2. The temperature is remarkably steady with less deviations from the straight line.

The record shown in Fig. 3 was one of the fastest observed, and the recorder pen travelled at a speed higher than 10° F./sec. Since this is the maximum speed obtainable from the amplifier and recorder it is, of course, possible that the actual rate of change was even faster. The temperature record was taken at the time of starting up the plant. The temperature rose almost instantaneously to 163° F. and exceeded the recording range at 164·5° F., on two occasions it fell almost instantaneously to 163 and 160° F.

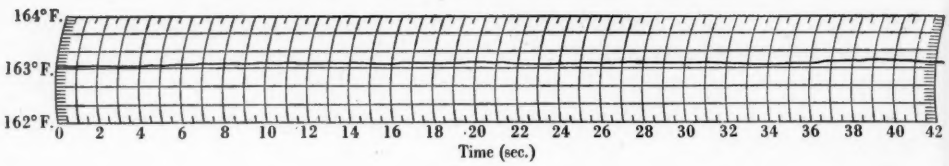

Fig. 2.

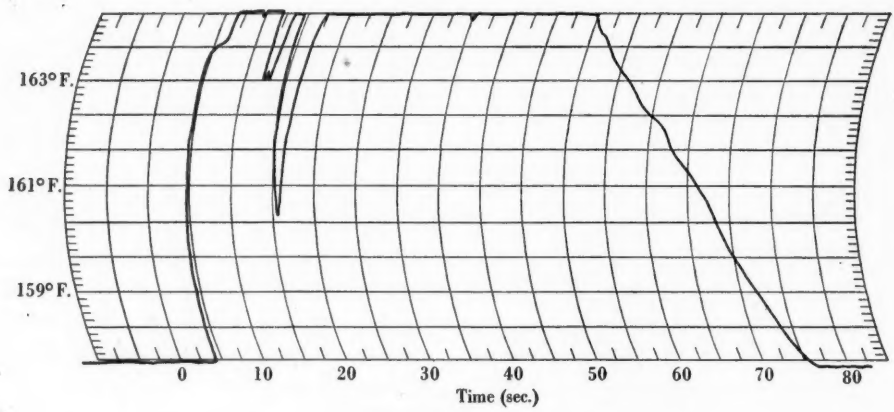

Fig. 3.

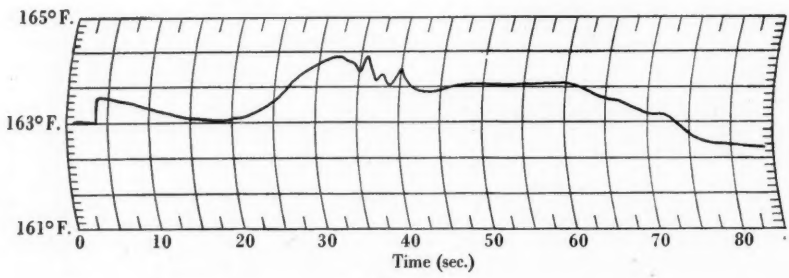

Fig. 4.

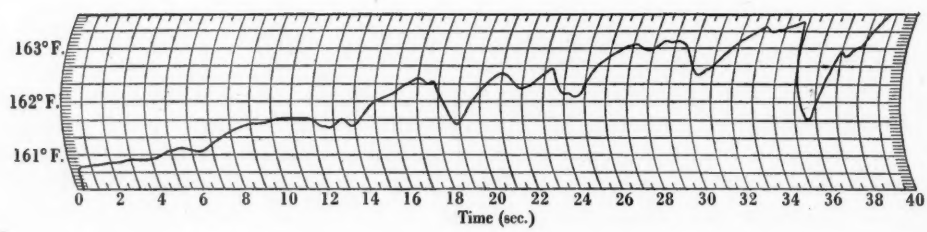

Fig. 5.

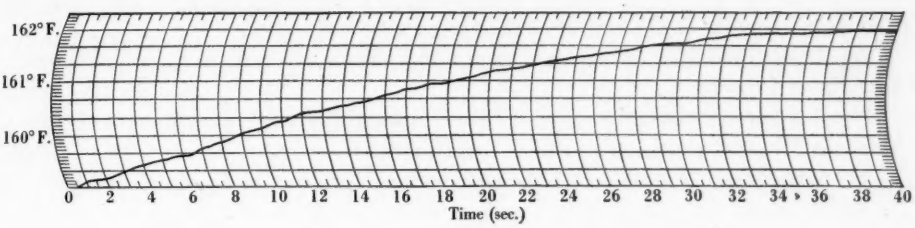

Fig. 6.

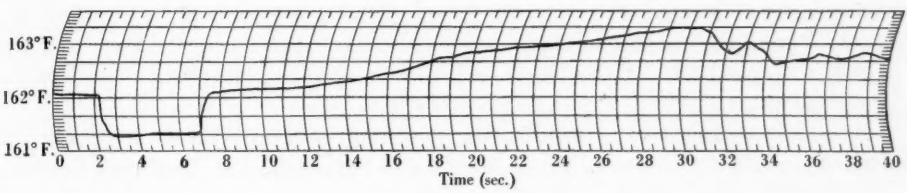

Fig. 7.

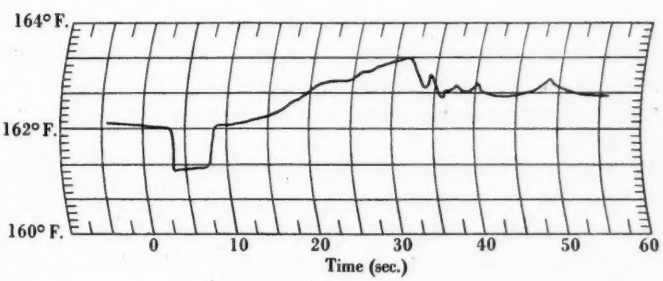

Fig. 8.

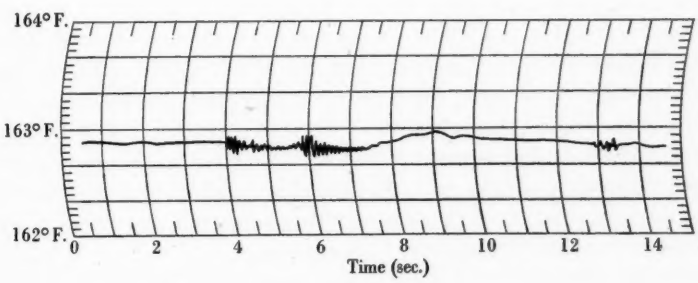

Fig. 9.

respectively. In two cases the pen travelled so fast that it could be observed only with difficulty by the naked eye. Shortly afterwards the temperature again fell below 158° F. and later recovered to the correct control temperature of 162° F.

Fig. 4 shows the temperature changes during a change of filter which was deliberately carried out very slowly.

Fig. 5 is another record obtained after the pasteurizing plant was put into operation immediately after a stoppage caused by switching off the pump motor; the rate of change in the 35th second is so high that the recorder was operating at full speed and the rate of change of the temperature might well be faster than 10° F./sec.

Fig. 6 shows, by way of contrast, an ordinary rise of temperature, which, in perhaps three out of four cases, is perfectly smooth and uninterrupted.

Fig. 7 shows the sequence of events after a rapid change of filter. At 2·2 sec. the temperature dropped almost instantaneously to below 162° F., and the rate of change at that point again amounted to about 10° F./sec. with the recorder working at its highest speed. During the run the milk began to leak under pressure from the filter, and the operator switched over at the 7th second, the temperature rising very rapidly to its previous level.

By a lucky coincidence the record in Fig. 7 was duplicated, since the recorders *A* and *B* were then connected in series. Fig. 8 shows the record obtained on the second recorder, the calibration of which was different. The chart speed was in fact five times less rapid than that of Fig. 7.

The greatest care was taken to ascertain whether some of the records were influenced by freak effects, but no such case could be discovered. Since, however, the amplifier included a galvanometer, special attention was directed to vibration. Shaking of the panel into which the amplifiers were built (the panel was standing loosely on the floor) had little if any effect. It was, however, fortunate that at the time of the tests major alterations were in progress at the dairy, and the influence of pneumatic drills could, therefore, be studied.

Fig. 9 shows the effect of the operation of such drills on the concrete floor on which the panel was mounted. The drills were operated only a few yards from the panel. As can be seen from the record their effect is entirely different from any previously observed. In view of the intense vibration caused by such drills the effect is in fact very small. When the pneumatic drills were operated only 6–8 yards away no influence on the records was detected.

It seems that the rate of change in the milk temperature in H.T.S.T. plants is some ten times faster than has been thought. It may be that the true figure is even higher than the 10° F./sec. which has been found during the present measurements, because the instruments available were not inserted where the fastest speeds might be expected.

The causes of the very rapid temperature fluctuations recorded in this paper are apparently not known, but they will have to be taken into account in the lay-out of plant in the future. If an accidental maladjustment of the filter causing leakage in the plant can produce effects like those observed, the provision of high-speed temperature-measuring equipment for the operation of the flow-diversion valve will be necessary, even though all other possible causes of irregularity be eliminated.

The recording equipment used in the present test is a modification of that described by J. A. Hall(2). The speed of response is certain, to an accuracy of approximately 0·1 sec.

I wish to thank Mr D. C. Gall for his advice and my colleagues and staff at Tinsley (Industrial Instruments) Ltd. for their help with the tests on milk pasteurization plant, and especially the management and staff of Job's Dairy. Thanks are also due to Mr J. E. Caffyn of the National Institute for Research in Dairying and Mr J. A. Hall of the National Physical Laboratory for attending the trials, and for their many useful suggestions.

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637.132:614.484

347. THE DISINFECTION OF CONTAMINATED METAL SURFACES WITH HYPOCHLORITE SOLUTIONS

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(With 1 Plate, 1 Text-figure and 6 Charts)

The work described here was designed to determine the value of hypochlorites as disinfectants for dairy utensils and the extent to which they could safely be used to replace steam and boiling water under wartime conditions.

The general results of our findings and their bearing on the methods of applying hypochlorites have already been published with tentative methods for the use of sodium hypochlorite on the farm (1, 2). Some of the evidence for the statements in these publications is now presented. The rest of the evidence will be presented in a later bulletin. It was not possible at the time to make the observations exhaustive, and the methods of practical application are not perhaps the simplest and most economical which could be devised.

Since the observations of Andrewes & Orton (3) and of Halwerda (4) that the germicidal action of chlorine solutions is due to the HOCl formed when chlorine or a hypochlorite is added to water and that the quantity formed is a function of *p*H, the most important are those by Rudolph (5), Weber (6) and Weber & Levine (7), who give a clear picture of the true relationship between *p*H, concentration, and reaction velocity of solutions containing available chlorine.

We intended to work with bacteria on surfaces but expected, after reviewing the literature, to find that the *p*H of the solutions containing chlorine* would be the chief factor affecting their efficiency as germicides under these conditions. Preliminary tests using well-washed milk cans rinsed with solutions at various *p*H values were made. The results differed little with changes in *p*H, whereas according to Weber & Levine, using bacterial spores in suspension, solutions at *p*H 7 may be expected to act about 200 times as rapidly as similar solutions at *p*H 10.

It can be inferred from the work of Myers & Johnson (8) that for the destruction of bacteria on surfaces the *p*H of solutions containing chlorine is of less importance than for bacteria in suspension. These workers sprayed infected milk bottles with trade brands of hypochlorites and found only very small differences in the efficiency of the solutions, though chloramine-T was much the slowest. These authors did not record the *p*H of their solutions, but, from the composition of the products used, there were undoubtedly marked differences in the *p*H of the diluted solutions.

But C. K. Johns (9), working with bacteria in wet films on glass slides and also with artificially infected milk bottles, obtained good agreement with expected results, i.e. the lower the *p*H the more efficient the chlorine solutions. Tests on a commercial milk plant were much less definite; samples from a bottle-filler gave exceptionally high counts

* The word chlorine is, in this paper, used to denote 'available chlorine' as determined by titration with sodium thiosulphate in the presence of acetic acid and iodine.

after using a solution at low *p*H, but as tests were not made with solutions of the same concentration of chlorine it is unsafe to draw conclusions.

Devereaux & Mallmann (10), working with a commercial milk plant, took samples of the chlorine rinse and samples of a water rinse after the chlorine rinse. They found that 11 p.p.m. chlorine was as effective or nearly as effective as 77 p.p.m. Again, there is no mention of *p*H, but undoubtedly from what we know of commercial hypochlorites, the solution with the lower concentration would have the lower *p*H. These workers regarded as satisfactory a residual count of 6 organisms per ml. of the solution (with thiosulphate) which had passed through the plant; the solution (75 gal.) in these tests was in contact with the plant for about 7 min. We suggest two explanations of their results: (1) that their plant was exceptionally clean and therefore the *p*H of the solution was an important factor, or (2) that they were estimating the residual flora only, most of the organisms having been destroyed in a few seconds even by the weakest solution. In the chemical disinfection of surfaces, though the vast majority of organisms may be destroyed in < 1 min., a few, the residual flora, may persist in the solution or in films and crevices for much longer.

Laboratory experiments with hypochlorites

Development of methods

It is not easy to obtain reproducible results using dairy utensils for tests of germicidal action, particularly if small or relatively small differences are to be determined. There is not only the question of uniform infection but also that of a reliable method of recovering the organisms, together with the difficulty of obtaining uniform surfaces. Bottles are not suitable because of their shape, and they are not representative of dairy utensils. Shallow tinned trays were therefore considered to be the most suitable.

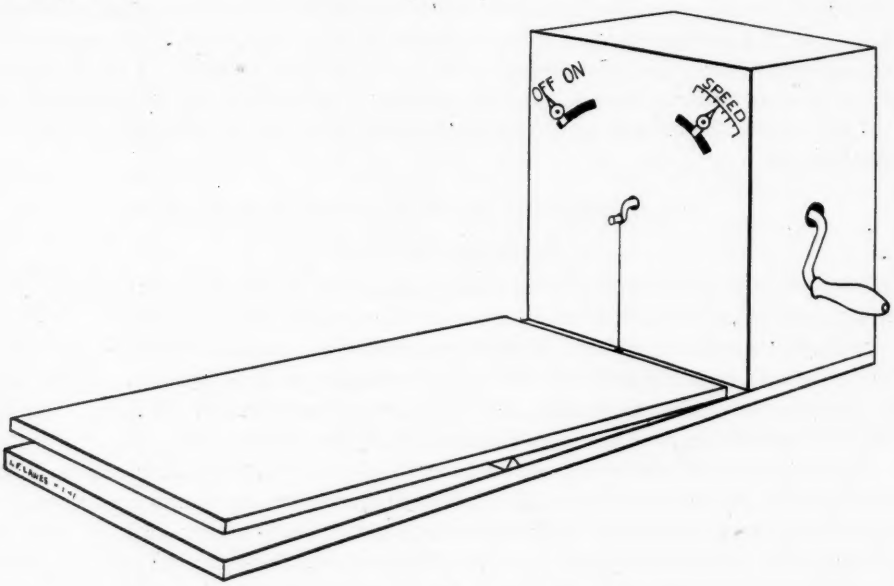
Trials were made with organisms of twenty types to determine their resistance to drying in the absence of organic matter (although it was our intention to use a standard quantity of organic matter in the final method) and the ratio of single cells to pairs or clumps after making suspensions by a standard method. One-tenth ml. of an aqueous suspension was put into a sterile Petri plate and spread by means of a wire loop. The plates were then exposed in a 37° C. incubator of low humidity for 1–24 hr. Drying was complete in about 30 min. Plates were poured with milk agar, incubated, the surviving organisms counted and the numbers compared with those on control plates. Little difference was found between the resistance of organisms grown for 24 and for 48 hr. on nutrient agar or between drying for 1 and 2 hr.

Micrococcus luteus (Schröt) Cohn, National Collection of Type Cultures, no. 2680, was the most resistant to drying, and resisted 2 hr. drying at 37° C. It was also most resistant to emulsification, i.e. to the breaking up of pairs and tetrads. Because of this tendency to form tetrads we decided to use the next most resistant organism for our experiments, i.e. *Staphylococcus aureus*, the 1933 'Insecticide strain' of Dr Reddish (National Collection of Type Cultures, no. 4136). With this organism 50% of the cells were non-viable after 1–2 hr. drying in the absence of organic matter.

Various methods for preparing suspensions were tried, and the simplest proved, on the whole, to be the most reliable. It was relatively easy to obtain suspensions free from clumps, but the ratio of single cells to pairs was about 4 : 1. This ratio could be increased to 16 : 1 or more by passing the suspension through a household cream emulsifier five

times, providing there was at least 10% milk present.* This treatment, oddly enough, did not increase the number of viable cells as estimated by plate counts.

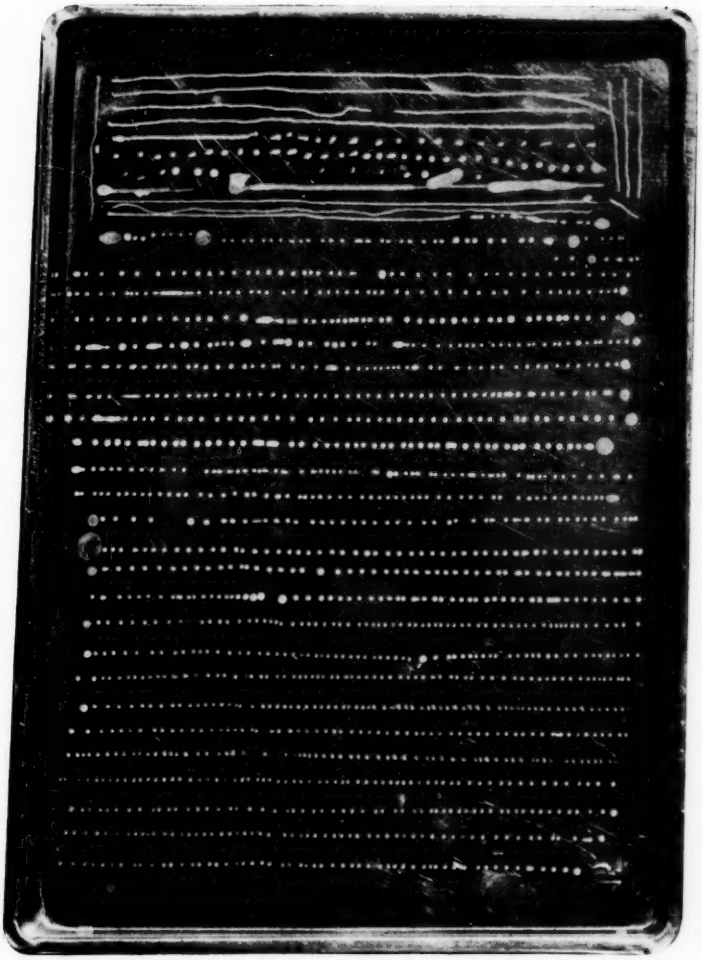
In practice it is almost certainly true to say that washed utensils are never free from foreign film, i.e. never clean in the chemical sense. The quantity and thickness of films vary greatly, but we wished to obtain a suitable and reproducible film of the thickness and extent to be found on farm dairy utensils. This might be approximated by smearing 1 ml. of a 9% whole-milk suspension over an area of about 1 sq.ft. Much more reproducible results and more survivals were, however, obtained by putting the suspension on the tinned trays in droplet form, and drying before use (see photograph of tray, Pl. 1).



Text-fig. 1. Apparatus for mechanical agitation of metal trays.

Except where otherwise stated agitation of the chlorine solutions on the infected trays was provided by mechanical means. The tray under test was placed centrally on a board having a central fulcrum consisting of a metal rod of 0·25 in. diameter. The board was attached loosely at one end to a cam ($\frac{1}{8}$ in.) on a revolving axle (see Text-fig. 1). The speed of the axle could be regulated. The rate of agitation was expressed as the number of times the wave of liquid traversed the tray and returned in 1 min., i.e. the number of to and fro movements. This was normally 18, and was sufficient to keep the liquid in continuous motion. The volume of liquid was such as to ensure that every part of the tray was consistently covered with liquid. The necessity for agitation, apart from its obvious beneficial effects in loosening material, was shown by numerous preliminary tests. There was a sharp decrease in the rate of destruction of the organisms on the trays as soon as movement of the chlorine solution was stopped.

* In the absence of organic matter there was a very pronounced reduction in the number of viable organisms caused presumably by the oligodynamic action of the copper from the brass plunger.



Preparation of bacterial suspension

The method of preparing the 9% whole-milk suspension of *Staph. aureus* was as follows: The organism was carried on dry nutrient agar slopes from day to day, and the growth after 48 hr. at 37° C. used for the suspensions. A standard loop of 1·5 mm. diameter was used for measuring the volume of cells, and when this was filled with the growth, and the excess removed by rubbing on the sides of the test-tube, it contained a remarkably consistent number of organisms: 1000–3000 million viable cells. Five of these loopfuls were added to 5 ml. of ¼ strength Ringer's solution, each loop of growth being moistened with the diluent and 'rubbed up' on the side of the 25 ml. McCartney bottle. One to two minutes were spent in bringing each loopful into suspension, and when this was done by an experienced person few or no clumps were to be seen under the microscope. At this stage the opacity was checked. The suspension was finally shaken vigorously for 10 min. The use of glass or steel balls or filtration through paper at this stage did not appear to be advantageous. Indeed, as would be expected when beads were used, particularly glass beads, the alkalinity and opacity of the suspension increased considerably.

One ml. of the above suspension was added to each 10 ml. of a 10% milk suspension prepared with fresh bulk milk heated to about 200° F. and cooled. This suspension was normally kept below 45° F. before and during use.

Tinned trays and method of preparation for use

Shallow tinned trays 13 by 9 in. with an area of about 0·8 sq.ft. were used. New trays were washed thoroughly, left soaking in sour milk for 24 hr., washed and steamed so as to remove the grease or 'bloom' present. After each experiment the trays were washed and placed in a steam sterilizer for 20 min.

Trays were infected each with 1 ml. of the suspension by dabbing the surface in a consistent manner about 2000 times, using the same pipette on all occasions. After drying there remained a series of rows of small circular semi-transparent dried milk films of approximately 2 mm. in diameter, each containing about 100,000 bacteria, and occupying in all about one-twelfth of the surface of the tray.

The trays were inverted and dried in a large gas-heated (internally) incubator at 37° C. having a fan. They dried in about 20 min. but were left in for 2 hr. There was not a marked difference in the number of survivors between 1 and 2 hr. drying, but the films were somewhat more difficult to wash off after the longer period.

Treatment or disinfection of infected trays and recovery of organisms

The mechanical agitator was adjusted, except where otherwise stated, to give 18 to and fro motions of the liquid per min. This corresponded to 9 revolutions of the axle of the motor per min.

An arbitrary volume of 400 ml. of the chlorine solutions was used for each tray, this being sufficient to keep the whole surface continually covered during treatment. A solution was poured on to the tray at one corner generally kept free from film for this purpose, and a stop-watch set as soon as half the solution was on the tray. About 2 sec. were required to pour all the solution on to the tray.

A stop-watch was used for all timing, and at the end of fixed periods the solutions were rapidly poured off and 100 ml. of sterile 0·2% sodium thiosulphate poured on. The

trays were squeegeed thoroughly with a 4 in. rubber squeegee, going over the whole surface 6 times.* The thiosulphate rinse was finally collected in a sterile bottle.

For a determination of the number of bacteria in the chlorine solutions themselves, at the time they were poured off the trays, the solutions were poured into sterile flasks containing excess sodium thiosulphate dissolved in 2–5 ml. of sterile water, or if the number of bacteria surviving in the chlorine solutions after a fixed period of time was required, the concentrated thiosulphate solution was added to the flasks of chlorine solution at this time.

Bacteriological methods

The thiosulphate rinses were shaken 100 times and bacteriological tests made by plating two serial dilutions in duplicate. Plates were incubated for 48 hr. at 37° C. The number of bacteria recovered from trays was determined from the mean number of colonies found on the plates, and when the number of colonies was <20 or >700 this has been noted. The agreement with expectation between the number of colonies on serial plates was usually good.

We have found that, in general, even if utensils have contained sour milk, provided that they have been well washed there will be less than 5,000,000 bacteria per square foot left on their surfaces. It is desirable therefore to aim at 99 or even 99·9% reduction in the flora in order to conform to reasonable bacteriological standards of 5000 per sq.ft. or per gallon of capacity. To ensure sufficient survivals for accurate estimations after a 99·9% reduction, it was necessary to use heavy infections of our trays, 100–300 millions. Counts below 3000 per tray could only be estimated roughly.

Table 1. *Attempt at the further dispersion of organisms by the addition of an organic wetting agent to the rinses from trays*

Sulphonated Lorol (93% sodium dodecyl sulphate) was added to 10 ml. portions of the rinses. The suspensions were shaken 50 times, left for 15 min. and shaken again before testing.

Rinse from	Added wetting agent (%)	Time of contact (min.)	Mean colony count (per ml.)†	Growth after 48 hr. at room temp. (in 0·05 ml.)
Tray no. 12, 10. iv. 41, with 9% milk film	0	15	82,500	+++‡
	0·005	15	76,000	7
	0·01	15	58,000	0
	0·05	15	14,000	0
Tray no. 16, 10. iv. 41, with whole-milk film	0	20	9,000	+++
	0·005	20	10,000	+++
	0·01	20	10,000	+++
	0·05	20	2,000	1
				Mean colony count† after 16 hr. at room temp.
Tray C3, 2. v. 41, with 9% milk film	0	20	3,740,000	1,190,000
	0·001	20	2,880,000	3,340,000
	0·0025	20	1,280,000	1,380,000
	0·005	20	2,940,000	570,000

* Rinsing alone for 30 sec., i.e. the method generally used for utensils before 1939, recovered only 5–35% of the organisms recovered using a squeegee; and swabs, 1 wet and 1 dry roughly 50%.

† Mean of two parallel plates. All plates were from the same dilution.

‡ +++ = uncountable.

The number of survivors on treated trays is expressed, in most cases, as the number of survivors per million of those found on the untreated or control trays. The loss or failure to grow caused by drying was in the region of 25%.

The organisms in the dried particles of milk removed from the trays were apparently released quite readily, since the particles generally dissolved without difficulty in the thiosulphate solution. Some tests were made with anionic wetting agents, but the substances used had generally little effect in increasing the number of colonies recorded and were often germicidal, as would be expected, at the *p*H and concentrations used. The figures in Table 1 are an example of one such test at *p*H 6·8. All solutions were shaken equally thoroughly before and after standing at room temperature.

The organisms survived quite well in the 0·2% thiosulphate rinses. Bacteriological tests were frequently repeated after the rinses had stood an hour on the bench, and the estimated number of survivors varied from plus 20% to minus 20% of the numbers found by the first test though very occasionally they were doubled, presumably because of insufficient shaking before the first test (see Table 2).

Hypochlorite solutions used

Except where otherwise stated all disinfectant solutions were prepared with sodium hypochlorite (Hopkins & Williams) sold as a pale yellow-coloured solution containing about 14% available chlorine and about 0·5% NaOH. When diluted with distilled water to 200 p.p.m. available chlorine the *p*H was 9·3–9·9. When hard water was used, the *p*H was 8·3–8·5, but the very soft Glasgow city water gave a *p*H of 9·7. A solution containing 200 p.p.m. chlorine is therefore very weakly buffered. Other brands of sodium hypochlorite gave much the same picture, while the *p*H differences were even greater when calcium hypochlorite was added to water of different hardnesses.

For use, fresh solutions were prepared daily, the available chlorine was determined by titration and the *p*H by glass electrode. These *p*H determinations have not been corrected (11, 12).

Local tap water was used for diluting the stock solution and, compared with boiled distilled water, had a chlorine demand of about 2 p.p.m.

When titrations were made in the presence of the alkali buffers which were often added to the chlorine solutions, chemically equivalent amounts of acetic acid were added in addition to the normal amount.

The loss of chlorine during use on the infected trays was only 2–3 p.p.m. after 5–15 min. exposure, whether in daylight or in darkness.

Variability of the method of recovering organisms from infected trays

A. *Untreated or control trays*

Eight trays were infected, dried and the organisms recovered, as previously described, in 100 ml. of 0·2% sodium thiosulphate. The number of organisms found on each tray is shown in Table 2, together with the standard deviations of the mean numbers on the trays. These deviations, whether obtained from the whole numbers or from the logarithms of the numbers, are surprisingly satisfactory, even when replicate tests were not made on the rinsings from each tray.

Table 2. *Variation in the number of organisms recovered from untreated (control) trays on the same date*

Tray	Bacteria on duplicate plates (1/10,000 dilution)	Millions per tray	Repeat test on rinsing from tray after 1½ hr. at 15° C.
1	96 100	98	116
2	58 50	54	113
3	110 137	123	128
4	115 116	115	115
5	99 100	100	—
6	94 115	104	—
7	113 118	115	—
8	(86) 95	95	—

	Mean	S.E.M.	S.D.	C.V.
Using whole numbers	100	8	21	21
Using logarithms	7·991	0·041	0·116	1·45

(86) = 86 +, as a small area was covered with a spreader.

Table 3. *Reliability of two methods of determining the total number of organisms on untreated (control) trays. (Tests made by the same worker)*

Date	A* 3 tests on 1 tray	A* Mean count (millions)		B† 1 test on each of 3 trays	B† Mean count (millions)
7. v. 41	Tray 1*a* 313‡			Tray 1*a* 313	
	b 443	326		2*a* 448	407
	c 223			3*a* 459	
	Tray 2*a* 448				
	b 231	295	Combined mean 319 (i.e. mean of 9 counts)		
	c 207				
	Tray 3*a* 459				
	b 260	366			
	c 289				
8. v. 41	Tray 1*a* 263			Tray 1*a* 263	
	b 300	257		2*a* 290	225
	c 208			3*a* 123	
	Tray 2*a* 290				
	b 185	228	Combined mean 270		
	c 210				
	Tray 3*a* 123				
	b 413	324			
	c 436				
16. v. 41	Tray 1*a* 225			Tray 1*a* 225	
	b 326	250		2*a* 198	219
	c 201			3*a* 235	
	Tray 2*a* 198				
	b 66	175	Combined mean 197		
	c 260				
	Tray 3*a* 235				
	b 115	165			
	c 145				

* On dilutions made from rinsing of tray—dilutions replicated thrice.

† Same trays as in column A. ‡ Each result the mean of two or three parallel plates.

Table 4. *Estimates of the mean number of organisms on control trays on any one date (in millions)*

Estimate based on	7. v. 41		8. v. 41		16. v. 41	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
3 trays, each the mean of 3 replicate tests	319	12·5	270	28·5	197	27
3 trays, 1 test on each	407	—	225	—	219	—
1 tray, mean of 3 replicate tests	326	—	257	—	250	—
1 tray, mean of 3 replicate tests	295	—	228	—	175	—
1 tray, mean of 3 replicate tests	336	—	324	—	165	—

In assessing the number of organisms on control trays on any one date, a mean of two or three tray counts was at first used. This method was wasteful, and it was therefore decided to attempt to use one tray only, using the mean of three replicate bacteriological tests of the rinsing from one tray.

Three replicate tests were made on the rinsings from each of three trays and the experiment was repeated on 3 days. These findings are presented in Tables 3 and 4.

It appears evident from the tests on three different dates that the mean of three replicated tests on the rinsing from one tray is likely to be just as reliable an estimate of the true mean as the mean of single tests on each of three trays.

An analysis of variance of the results obtained on the first date showed no significant difference (using logarithms) at the 5% level between trays or replicates, and all but the result 407 fall within twice the standard error of the combined mean. The latter result is >4·3 times the standard error.

The reason for the great variation between the results of replicated tests with the rinsing from a single tray is not fully understood. The work was done by the same worker who did the tests shown in Table 2, and all counts were made on plates from the 1/10,000th dilution. There was good agreement between parallel plates and with expectation between plates from serial dilutions.

Subsequent estimates of the number of organisms on control trays were based on the mean of three replications of the rinsing from one tray.

B. *Experimental or treated trays*

Considerably greater variation would be expected between treated trays on the same date than between control trays. To estimate this variation twelve trays were exposed for 1 min. to 200 p.p.m. available chlorine at *p*H 10 and another six trays to the same treatment except that instead of soda ash a mixture of caustic soda and soda ash was used as the buffer.

Half the trays were rinsed and tested by one worker and the remainder by a second worker.

The results and analyses are given in Table 5, and by a comparison with Table 2 it will be seen that the variation between parallel experimental trays is about three times as great as that between control trays. With the number of trays used there was no significant difference between the results obtained by the two different workers.

The difference between the number of bacteria recovered from trays treated similarly may be attributed to numerous factors not easily controllable. A few large drops occurring accidentally were seen to be associated with larger recoveries of bacteria. As far as could be judged by visual inspection the amount of film removed during treatment was fairly

constant at approximately 20% of the total. The variation in any stated time of exposure was not more than 5 or 6 sec.

Table 5. *200 p.p.m. available chlorine at pH 10. Trays exposed for 1 min.*

A. Variation between test trays on the same date and between two workers.
B. Variation between alkalis.

Tray no.	*p*H	% alkali	Alkali mixture	Survivors per tray	Survivors per million	
Untreated tray				Mean 107,000,000		
1	9·96	0·125		29,000	271	
2	9·96	0·125		19,000	178	Worker A, trays 1–6, mean log = 4·450. Worker B, trays 7–12, mean log = 4·599. Difference not significant by pooled variances; $p = c.\ 0{\cdot}5$
3	9·96	0·125	Soda	28,000	252	
4	9·96	0·125	ash	23,000	215	
5	9·96	0·125		101,000	944	
6	9·96	0·125		14,000	131	
7	9·96	0·125		30,000	280	Trays 1–12, mean log = 4·524. S.D. = 0·309. C.V. = 6·83
8	9·96	0·125		14,000	131	
9	9·96	0·125	Soda	53,000	495	
10	9·96	0·125	ash	27,000	252	
11	9·96	0·125		133,000	1,245	
12	9·96	0·125		49,000	458	
13	10·06	0·05		77,000	720	Trays 13–18, mean log = 4·828. S.D. = 0·298. C.V. = 6·2
14	10·06	0·05		36,000	336	
15	10·06	0·05	Na OH 0·01	105,000	981	
16	10·06	0·05	Soda ash 0·04	64,000	598	
17	10·06	0·05		148,000	1,383	
18	10·06	0·05		34,000	318	

Difference between 0·125 and 0·05% alkali not significant. $t = 1$, whereas for 5% level $t = 2{\cdot}12$.

Variation in results from day to day

We were not able to control the method sufficiently well to enable us to predict the deviations in results from day to day; they might be practically identical or show extreme variation.

The results of tests made on two or more substances on the same date are comparable, as are the mean results of a series of such tests made on a number of different dates. A direct comparison cannot be made, however, with the same degree of accuracy between the results of tests on two substances if the tests are made on different dates. For instance, the mean number of organisms recovered from six trays exposed for 1 min. to 200 p.p.m. available chlorine at *p*H 10 on the same date was 400 bacteria per million, whereas the mean number recovered from six trays tested on six different dates was 2800, both with approximately the same standard deviation.

The latter type of data was occasionally used, however, if the mean was within twice the standard error of the mean of a series of comparisons made on the same dates.

The amount of dried film washed off the trays by the standard treatment with water and alkali solutions alone

Trays were flooded with 400 ml. of various solutions containing no hypochlorite, and the amount of film washed off in a specified time was determined by visual inspection of the films during treatment and by a determination of the number of organisms remaining on the trays after treatment.

The alkalis were chosen not so much for their detergent qualities as for their buffering properties. It was desirable, also, to use the common alkalis.

The results obtained are shown in Table 6, and the findings of tests on the same solutions containing bacteria in suspension are given in Table 7.

Table 6. *Number of bacteria removed from trays by water and alkali solutions alone (no hypochlorite added)*

Duplicate trays at ½ and 4 min. exposure. Temp. 62° F. All tests of the same date.

Tray no.	Treatment	pH	Exposure time (min.)	% film washed off*	Bacteria surviving (millions)	Bacteria removed (%) No.	Mean
	Untreated tray†	—	—	—	145	—	—
1	Tap water	7·5	½	20*b*	84	42	34
2		7·5	½	20*b*	107	26	
3		7·5	4	25*b*	94	35	37
4		7·5	4	25*b*	89	39	
5	0·125% Soda ash	10	½	20*b*	82	43	32
6		10	½	20*b*	113	22	
7		10	4	20*b*	57	61	60
8		10	4	40*x*	59	59	
9	0·05% alkali, i.e. NaOH 0·03 Soda ash 0·02	11	½	30*b*	91	37	47
10		11	½	30*b*	63	57	
11		11	4	80*c* in 1 min.	43	70	68
12		11	4	70*c* in 1 min.	51	65	
13	0·5% alkali, i.e. NaOH 0·05 Soda ash 0·45	11	½	30*c*	46	68	64
14		11	½	20*c*	58	60	
15		11	4	90*c* in 1 min.	43	70	71
16		11	4	80*c* in ½ min.	41	72	
17	0·05% alkali, i.e. NaOH 0·049 Soda ash 0·001	11·5	½	30*c*	61	58	—
18		11·5	4	80*c*	64	56	—

* Removal of dried-milk films during exposure to alkali and/or hypochlorite solutions (by inspection).

a = Little or no removal of milk films. *b* = Chiefly submarginal material removed.

c = Chiefly centres of films only remaining. *x* = Various parts of film being removed at the same time.

The order of occurrence is generally *a*, *b*, and *c*; *x* is exceptional. In nearly every case, however, the base of the extreme outer margin remained as a faint visible ring, even, sometimes, after the tray was rinsed and squeegeed.

† Mean of three replicate tests, three parallel plates each, i.e. mean of nine plates.

Table 7. *Survival of bacteria in the water and alkali solutions used in the previous experiment (Table 6)*

Organisms added direct to solution at 61° F.

Solution (as Table 6)	pH	Exposure time (min.)	Mean no. of bacteria on duplicate plates	No. of bacteria expressed per 1 ml. of original suspension (millions)
Tap water	7·5	1	266	133
		5	255	128
0·125% alkali	10	1	290	145
		5	228	114
0·05% alkali	11	1	326	163
		5	320	160
0·5% alkali	11	1	331	166
		5	277	139
		15	333	167
0·05% alkali	11·5	1	280	140
		5	295	148
		15	263	132

The figures in Table 6 are, of course, too few to show any really accurate differences between the solutions used, though they are quite sufficient to demonstrate that the

detergent action itself has an influence, though not a major one, on the results reported later for hypochlorites. Even the best reduction shown, that with 0·5% alkali, is only about twice as great as with water alone, and after 4 min. contact there were still 42 million bacteria remaining on the trays, whereas as little as 12 p.p.m. available chlorine with no added alkali reduces the numbers by more than 90% in 2 min. Johns(9) found no difference in rinsing properties between distilled water and various alkaline solutions when using wet films.

From inspection of the trays during treatment it was noticed that there was little further removal of the film after the first ½–1½ min. exposure, and most of the bacteriological results support this observation.

The significance of pH in solutions containing chlorine for sterilizing metal surfaces

In the results described here it should be borne in mind that the organism *Staph. aureus* is in the dried films on the trays and not merely on the surface of the films; secondly, that the films are more or less removed during the application of the disinfectant (see Table 6); and thirdly, that the film is not impervious while on the tray or after removal, i.e. it becomes hydrated.

The tests described in this section were made with solutions containing 200 p.p.m. chlorine adjusted to *p*H 7, 10, 11 and about 11·5 and the results are shown in Tables 8–11, and Charts 1–3. For convenience, the numbers of surviving bacteria shown in the tables are expressed as numbers of survivors per million organisms recovered from control trays; the total survivors per tray are about 100 times as many as shown, depending on the numbers originally present on control trays, about 100–200 million. The logarithms of the number of survivors per million have been used for tests of significance and for estimating the regression coefficients.

The shapes of the curves obtained by plotting the logarithms of the survivors per million against the time of exposure in minutes for solutions at different *p*H (Chart 3) are not the same though the rate of destruction at each *p*H may, for practical purposes, be taken to assume that of a unimolecular reaction at *certain stages*.

Table 8. *The relationship between* 200 *p.p.m. available chlorine at* pH 7 *and* 10 (*data used in Chart* 1)

Date of exp.			8.v.41	4.vi.41	23.v.41	27.v.41	29.v.41	16.v.41	Mean log survivors per million	
Mean of control trays (millions) ...			270	110	93	150	97	197		
*p*H	Buffer (%)	Exposure (min.)	Bacteria surviving per million						1st 2 exps.	5 exps.
7 (6·61–7·16)	$NaH_2PO_4.2H_2O$, 0·2	½	4,890	57,300	27,000	15,300	13,200	—	4·224	4·237
		1	1,380	10,900	7,320	7,340	5,060	1,420	3·589	3·722
		2	585	1,360	1,040	253	382	—	2·951	2·781
		4	—	15	—	27	20	14	—	—
		6	—	—	38	—	—	—	—	—
		8	—	9	—	—	—	—	—	—

Date of exp.			8.v.41	4.vi.41	23.iv.41	29.iv.41	2.v.41	7.v.41	Mean log survivors per million	
Mean of control trays (millions) ...			270	110	266	115	370	320		
			Bacteria surviving per million						1st 2 exps.	6 exps.
10 (9·93–10·19)	Soda ash, 0·125	½	5,120	33,600	7,140	24,300	12,300	—	4·118	4·113
		1	412	3,270	240	3,220	5,950	3,820	3·065	3·229
		2	13	118	20	29	325	41	1·593	1·679

Table 9. *The relationship between* 200 *p.p.m. available chlorine at* pH 7, 11 *and* 11·5 (*see Charts* 2 *and* 3)

	Date of exp.		23. v. 41	27. v. 41	29. v. 41	4. vi. 41	Mean log survivors per million (4 exps.)
	Mean of control trays (millions)		93	150	97	110	
*p*H	Buffer %	Exposure (min.)	Bacteria surviving per million				
7	$NaH_2PO_4.2H_2O$, 0·2	½	27,000	15,300	13,200	57,300	4·374
(6·61–7·16)		1	7,320	7,340	5,060	10,900	3·868
		2	1,040	253	382	1,360	2·784
		4	—	27	20	15	1·303
		6	38	—	—	—	1·580
		8	—	—	—	9	0·954
11	NaOH, 0·03	½	7,740	14,500	4,230	33,600	4·051
(10·91–11·04)	Soda ash, 0·02	1	63*	289*	147*	910	2·347
		2	26	73	78	291	1·909
		4	—	32	14	—	1·326
		6	24	—	—	—	1·380
		8	—	38	11	—	1·311
11·5	NaOH, 0·048	½	344,000	487,000	289,000	100,000	5·422
(11·38–11·48)	Soda ash, 0·002	1	88,200	387,000	77,400	72,700	5·071
		2	2,370	7,340	825	4,460	3·452
		4	—	25	33	—	1·447
		6	18	—	—	—	1·255

* Mean of two or three trays.

Table 10. *The effect of the concentration of the alkalies used to buffer chlorine solutions at* pH 11. 200 *p.p.m. available chlorine. Trays exposed for* 1 *min.*

Date	% alkali	Alkali mixture	*p*H (glass E)	Survivors per tray*	Survivors per million	Mean same date	Mean all trays	Film washed off (%)
23. v. 41	0·05	0·03 NaOH, 0·02 Soda ash	11·03	6,800	73·1	62·4	179	50*c*
	0·05		11·03	4,800	51·6			40*c*
27. v. 41	0·05		10·91	54,000	360	389		30*c*
	0·05		10·91	39,000	260			30*c*
	0·05		10·91	37,000	246			30*c*
29. v. 41	0·05		10·94	15,300	158	147		30*c*
	0·05		10·94	14,300	147			30*c*
	0·05		10·94	13,300	137			30*c*
23. v. 41	0·5	0·05 NaOH, 0·45 Soda ash	10·97	16,000	172	307	516	40*c*
	0·5		10·97	41,000	441			40*c*
27. v. 41	0·5		10·9	114,000	761	828		30*c*
	0·5		10·9	147,000	980			30*c*
	0·5		10·9	110,000	734			30*c*
29. v. 41	0·5		10·94	52,000	536	520		30*c*
	0·5		10·94	16,000	165			30*c*
	0·5		10·94	33,000	340			30*c*

* Only those of the same date are strictly comparable. *c*. See note to Table 6.

The reaction velocity of solutions (200 p.p.m.) at *p*H 11 and 11·5 is apparently nearly constant during the period of 99·9% destruction, while that of solutions at *p*H 7 and 10 is very approximately the same as solutions at *p*H 11 during the period of 95% destruction, after which it slows down.

From Table 8 and Chart 1 solutions at *p*H 10 appear to be more rapid in their disinfectant action than those at *p*H 7. This is probably true, *with the buffers used*, but the data are insufficient to show a significant difference; $t = c.1{\cdot}4$ with 28 degrees of freedom.

Solutions at *p*H 11·4–11·5 were, however, significantly less germicidal (at the 1% level)

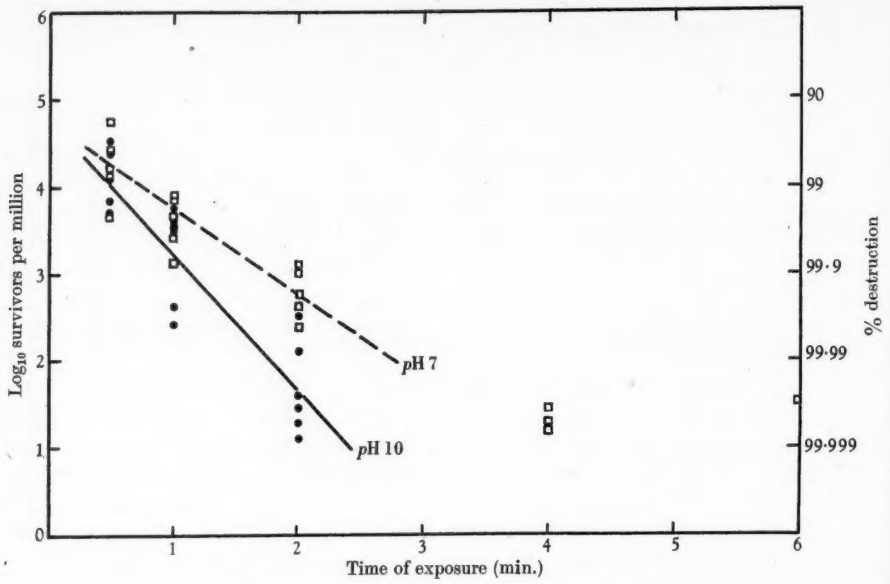


Chart 1. The relationship between 200 p.p.m. available chlorine at *p*H 7 and at *p*H 10. Temperature 16° C. The regression coefficients are not significantly different. [The lines have, of course, the same origin, i.e. log. 6. For convenient reference, the percentage destruction is shown on the right of the graph.]

*p*H 7: $b = -0{\cdot}966 \pm 0{\cdot}047$; $a = 4{\cdot}71$ (5 trials) *p*H 10: $b = -1{\cdot}609 \pm 0{\cdot}092$; $a = 4{\cdot}88$ (6 trials)

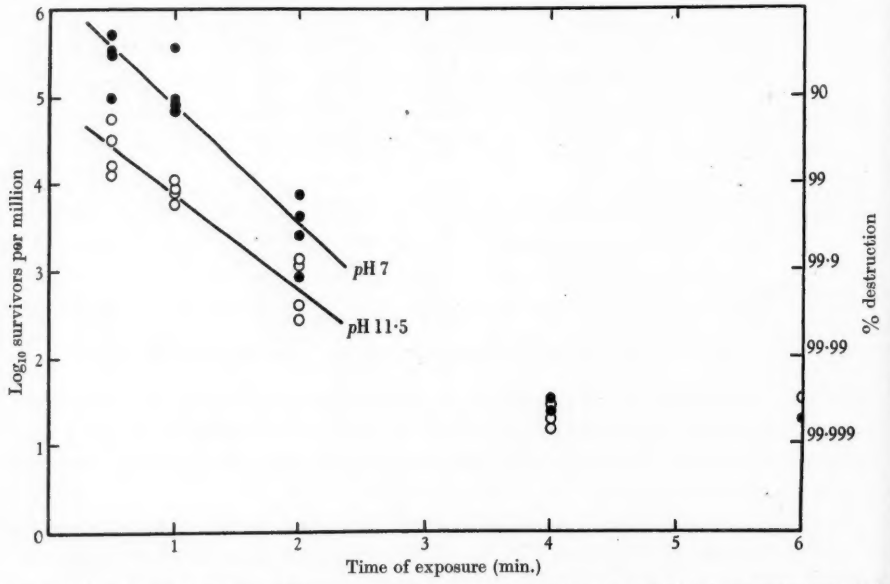


Chart 2. The relationship between 200 p.p.m. available chlorine at *p*H 7 and at *p*H 11·5. Temperature 16° C. The regression coefficients are significantly different.

*p*H 7: $b = -1{\cdot}062 \pm 0{\cdot}031$; $a = 4{\cdot}91$ (4 trials) *p*H 11·5: $b = -1{\cdot}36 \pm 0{\cdot}064$; $a = 6{\cdot}23$ (4 trials)

than solutions at *p*H 7 (Chart 2), and there is no doubt that at *p*H 11 the reaction velocity was greater than at either *p*H 7 or 11·5 (Chart 3).

The most rapid destruction of 99·9% of the organisms was with 200 p.p.m. chlorine at *p*H 11. The average times for 99, 99·9 and 99·99% destruction obtained from Chart 3 were 0·5, 0·8 and 1·8 min. respectively, and the approximate mean germicidal time equivalents at *p*H 7, 10 and 11·5 were as follows:

	*p*H			
% killed	7	10	11	11·5
99	1·7	1	1	3·3
99·9	2·3	1·5	1	3
99·99	1·5	1	1	1·8

These germicidal time equivalents may be compared with those of Weber(6) and Weber & Levine(7) for 99% destruction of spores in suspension (see Table 23).

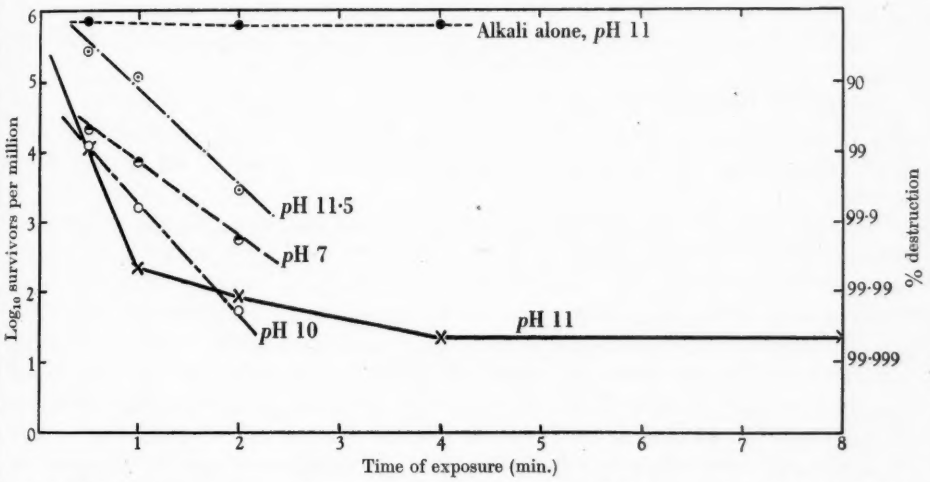


Chart 3. The relationship between 200 p.p.m. available chlorine at *p*H 7, 10, 11 and 11·5 and 0·5 % alkali at *p*H 11 with no available chlorine. All lines fitted by method of least squares except that for *p*H 11. All points are the mean of several trials.

The velocity constant, k, for 200 p.p.m. chlorine at *p*H 11·5 with the buffers used was $3{\cdot}1 \pm 0{\cdot}15$, and at *p*H 11 about 9 (Table 9). It seems very probable, however, that if a weaker buffer had been employed the solution at *p*H 11·5 would have had much the same velocity as at *p*H 11 (see Discussion below). There was detectably less film removed by solutions at *p*H 7 than by those above this *p*H (see Tables 12 and 13).

Discussion, and the influence of alkalis and their concentration

The probable reason why solutions at *p*H 7 are not much more rapid than solutions at *p*H 10 or 11 is that bacteria are destroyed in the film before hydration has advanced sufficiently to raise the *p*H of the film appreciably. Providing the solutions are not too strongly buffered that portion which is wetting and being absorbed by the film will have a *p*H very similar to that of the film itself. Most films, as in our case, will be at *p*H 7 or

lower, and because of the organic matter present, particularly milk protein, will have a high buffering capacity.

Probably the more tenacious and less readily hydrated the film the greater the local buffering effect and the less important the *p*H of the chlorine solutions. The results obtained with a film which is easily removed and readily hydrated will be nearer those obtained with suspensions of bacteria, i.e. the number of surviving bacteria will be more directly related to the *p*H of the chlorine solutions (see Johns(9)).

Costigan(14) has shown that the addition of 1 part of a milk-broth culture to 10 parts of two commercial hypochlorite solutions, diluted to contain 200 p.p.m. available chlorine, reduced the *p*H of one from 10·3 to 8·3 and of the other from 8·85 to 7·5.

We have found that milk of *p*H 6·66, to which sufficient sodium hypochlorite was added to give an initial concentration of 250 p.p.m. available chlorine had a *p*H of 6·69–6·73.* When soda ash was incorporated to the extent of 0·25%, without diluting the milk more than 1%, the suspension had a *p*H of 7·7. With the same amount of sodium metasilicate the *p*H of the mixture was 8·96. Higher concentrations of milk solids would undoubtedly lower the *p*H of the alkaline hypochlorite solutions still further.

Presumably the higher the *p*H of the solutions the more rapid the hydration of the film of the type used. This, apart from or in addition to the detergent action, is a possible explanation for the greater germicidal rate of the solutions at *p*H 11 than those at *p*H 7.

We have not yet tested the theory, but believe that the reason for the slower rate found at *p*H 11·5 is that the buffer used (Table 9) was too strong. A weaker buffer at the same *p*H or higher may have resulted in the solution being more rapidly germicidal. Some evidence for the suggestion is provided by the data in Table 10. The table gives the results obtained from exposing trays for 1 min. to chlorine solutions buffered at *p*H 11 with 0·05% alkali and with 0·5% alkali. We should expect, from previous experience and from the figures shown in Table 6, that any difference would be in favour of the stronger solution. The findings are, however, that the solution buffered with 0·05% alkali is significantly better than that buffered with 0·5% alkali; $t=4$† and for 7 degrees of freedom $p=<0{\cdot}01$.

Further data showing the tendency of increasing concentrations of alkali, at the same *p*H, to reduce the efficiency of chlorine solutions are given in Table 11 and Chart 4. The presence of some alkali or other detergent is obviously desirable, but its composition is important. Solutions having a *p*H of 11·4 or more are, as before, decidedly less germicidal, and these results suggest that the buffering power is not in fact the chief factor. In the solution with 0·25% NaOH, the alkali itself is germicidal at room temperature. It should also be noted that the solutions having a high *p*H were the better detergents, yet in spite of the fact that they removed twice as much of the dry film as solutions at a lower *p*H they left many more viable organisms on the trays (Table 11).

It is fully realized that our findings only apply to imperfectly cleaned utensils, and that chlorine solutions of low *p*H are certainly more rapidly effective than those of high *p*H in destroying bacteria on very clean surfaces and on highly hydrated surfaces.

* Sodium hydroxide was present in the stock hypochlorite solutions to the extent of 0·6–1·6%, and the amount present in the milk was therefore only 0·004 and 0·0016% respectively.

† Calculations based on numbers of bacteria surviving and not on logarithms of numbers.

Table 11. *Some effects of pH and concentration of alkali (pairs of trays on each of two dates)*

Table 11. *Some effects of pH and concentration of alkali (pairs of trays on each of two dates)*

Time 1 min. NaOCl 200 p.p.m. available chlorine. Temperature of solution 62° F.

Date	Alkali	pH	Film removed† (%)	Bacteria surviving on tray	Bacteria surviving per million			Bacteria surviving in solution*
					No.	Log	Mean	
21. iv. 41	Untreated tray	—	—	226,000,000	—	—	—	—
25. iv. 41	,,	—	—	265,000,000	—	—	—	—
21. iv. 41	No added alkali	8·98	20*b*	410,000	1,810	3·258		
		8·98	20*b*	262,000	1,160	3·064		
25. iv. 41	,,	8·95	20*b*	230,000	870	2·940	3·14	—
		8·95	20*b*	525,000	1,980	3·297		
21. iv. 41	¼% {0·135% $Na_2SiO_3.5H_2O$, 0·115% $NaH_2PO_4.2H_2O$	9·85	20*b*	280,000	1,240	3·093		
25. iv. 41		9·85	20*b*	341,000	1,290	3·111	2·82	Nil
		9·85	20*b*	46,000	174	2·241		
21. iv. 41	½% {0·25% $Na_2SiO_3.5H_2O$, 0·25% $NaH_2PO_4.2H_2O$	9·86	20*b*	1,200,000	5,320	3·726		
		9·86	20*b*	450,000	1,990	3·299		
25. iv. 41		9·9	20*b*	900,000	3,400	3·531	3·39	Nil
		9·9	20*b*	260,000	981	2·992		
21. iv. 41	¼% Soda ash	10·2	20*b*	36,000	159	2·201		
		10·2	20*b*	36,000	159	2·201		
25. iv. 41	,,	10·2	20*b*	110,000	415	2·618	2·49	*c.* 1,000
		10·2	20*b*	230,000	868	2·939		
21. iv. 41	1% Soda ash	10·3	Nil ?	152,000	673	2·828		
		10·3	Nil ?	47,000	208	2·318		
25. iv. 41	,,	10·3	20*b*	103,000	389	2·590	2·67	Nil
		10·3	20*b*	237,000	895	2·952		
21. iv. 41	¼% $Na_2SiO_3.5H_2O$	11·44	40*c*	>5,000,000	—	—		
		11·44	40*c*	>5,000,000	—	—		
25. iv. 41	,,	11·44	40*c*	60,800,000	230,000	5·784	5·50	370,000
		11·44	40*c*	45,200,000	170,000	5·230		
25. iv. 41	$\frac{1}{16}$% NaOH	11·9	40*c*	173,000,000	653,000	5·815		
		11·9	40*c*	145,000,000	547,000	5·738	5·78	380,000
21. iv. 41	¼% NaOH	11·95	40*c*	>15,000,000	—	—		
		11·95	40*c*	>15,000,000	—	—		
25. iv. 41	,,	11·92	40*c*	39,000,000	149,000	5·173	5·08	*c.* 500
		11·92	40*c*	25,000,000	95,500	4·980		

* Solution poured off trays at end of 1 min. direct into sterile flask containing sodium thiosulphate (dissolved in 2–3 ml. sterile water) to neutralize the chlorine. Numbers refer to total bacteria recovered in the 400 ml. of solution. Nil = <400.

† See footnote to Table 6, p. 33.

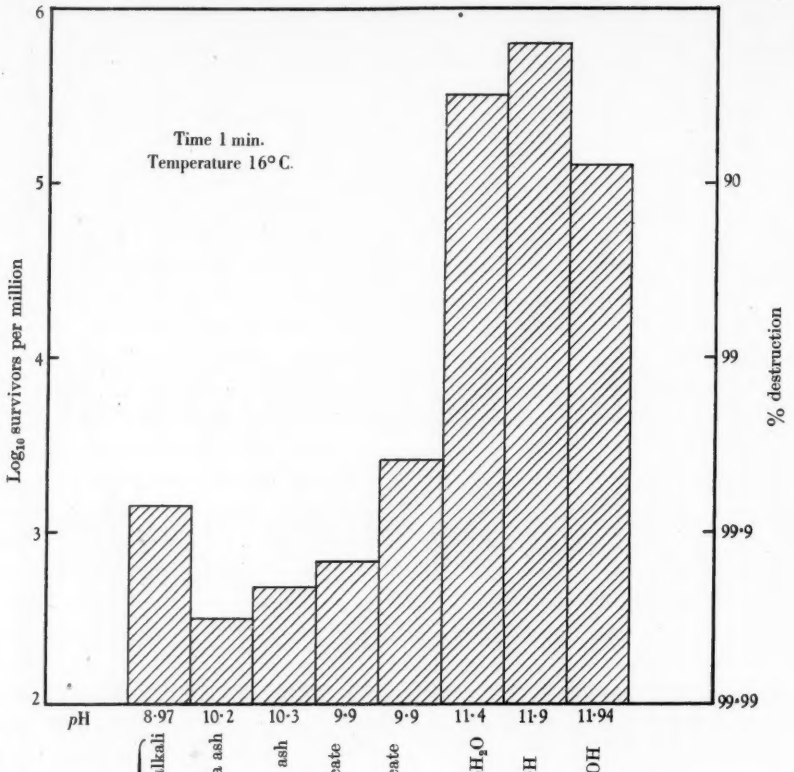


Chart 4. The effect of alkali and *p*H on the destruction of *Staphylococcus aureus* by a solution containing 200 p.p.m. available chlorine. (See Table 11.)

Table 12. *The influence of* p*H on the germicidal power of* 200 *p.p.m. available chlorine at* 60° F. *on* 27 *May* 1941

Tray no.	*p*H	Concentration of alkali (%) NaOH	Soda ash	Total	Exposure time (min.)	Film removed† (%)	Bacteria surviving per tray	Bacteria surviving per million	Bacteria surviving in the chlorine solution*
Control					0	—	150,000,000	—	—
1	6·98	(0·2% $NaH_2PO_4.2H_2O$)			½	20*b*	2,300,000	15,000	0
2					1	20*b*	1,100,000	7,300	—
3					2	20*b*	38,000	250	0
4					4	20*b*	4,000	27	—
5	10·91	0·03	0·02	0·05	½	30*c*	2,180,000	14,500	0
6					1	30*c*	54,000	360	0
7					1	30*c*	39,000	260	—
8					1	30*c*	37,000	246	—
9					2	30*c*	11,000	73	0
10					4	30*c*	4,800	32	0
11					8	30*c*	5,800	38	—
12	11·38	0·048	0·002	0·05	½	30*c*	73,000,000	487,000	3,000,000
13					1	30*c*	58,000,000	387,000	430,000
14					2	30*c*	1,100,000	7,340	4,400
15					4	30*c*	3,700	25	0
16	10·9	0·05	0·45	0·5	1	25*c*	114,000	761	—
17					1	25*c*	147,000	980	—
18					1	25*c*	110,000	734	—

* 10 sec. after pouring the solution off the trays. Number per tray.
† See footnote, Table 6, p. 33.

Table 13. *The influence of* pH *on the germicidal power of* 200 *p.p.m. available chlorine at* 60° F. *on* 29 *May* 1941

Tray no.	pH	Concentration of alkali (%) NaOH	Soda ash	Total	Exposure time (min.)	Film removed† (approx.) (%)	Bacteria surviving	Bacteria surviving per million	Bacteria surviving in the chlorine solution
Control					0	—	97,000,000	—	—
1	6·9	(0·2% $NaH_2PO_4.2H_2O$)			½	20*b*	1,280,000	13,000	0
2					1	20*b*	490,000	5,100	—
3					2	20*b*	37,000	382	—
4					4	20*b*	1,900	20	—
5	10·94	0·03	0·02	0·05	½+	30*c*	410,000	4,230	0
6					1	30*c*	15,300	158	0
7					1	30*c*	14,300	147	—
8					1	30*c*	13,300	137	—
9					2	30*c*	7,600	78	—
10					4	30*c*	1,400	14	—
11					8	30*c*	1,100	11	—
12	11·46	0·048	0·002	0·05	½	30*c*	28,000,000	289,000	480,000
13					1	30*c*	7,500,000	77,400	290,000
14					2	30*c*	80,000	825	0
15					4	30*c*	3,200	33	0
16	10·94	0·05	0·45	0·5	1	30*c*	52,000	536	—
17					1	30*c*	16,000	165	0
18					1	30*c*	33,000	340	—

† See footnote, Table 6, p. 33.

Survival of Staphylococcus aureus *in the solution poured from trays*

It is obviously important to know that the organisms removed from a surface are quickly killed in the disinfectant. Tests have been made on the solutions poured off the trays using the technique described previously.

With the dried trays about 20–60% of the organisms leave the surface during the first 0·5 min. in contact with the chlorine solutions, and they continue to do so at a much less rapid rate. These organisms are more quickly destroyed, however, than those remaining on the trays, even when the chlorine solution is at *p*H 11·5.

Tables 12 and 13 show that with 200 p.p.m. chlorine at *p*H 7–11 the solutions tested were sterile within 10 sec. of pouring off the trays. At *p*H 11·4–11·5, however, only those solutions which had been on the trays for 2–4 min. were sterile, i.e. contained less than one organism per ml. of solution when tested within 10 sec. The data in Table 14, however, show that even in solutions at such a high *p*H the organisms were completely destroyed in 1 min. or less after 0·5 min. on the tray. The same table shows that at *p*H 10 solutions with 25 p.p.m. chlorine are not sterile after 4 min. on the trays, but those with 100 p.p.m. chlorine are sterile after 2 min. or less on the trays.

Even when the films containing the bacteria are easily removed the destruction appears to be more rapid in the solutions than on the trays, except possibly when a wetting agent is used and the moist film is exceptionally quickly washed off (Table 15).

There would appear to be little doubt that apart from exceptionally resistant organisms there will be few if any surviving bacteria in chlorine solutions containing 200 p.p.m chlorine after they have been in contact with an infected or contaminated surface for half a minute, providing the *p*H of the solution is not over 11.

Table 14. *Destruction of bacteria in the solutions on trays is more rapid than on the surface. Trays with dry films. Trays exposed to solutions at* pH 10–11·5

Chlorine in the solution	pH	Exposure time (min.)	Bacteria surviving: On trays	Bacteria surviving: In the solution poured off after an additional exposure of
No treatment (3 trays)	—	0	320,000,000	—
Water only	7·5	1	269,000,000	67,000,000 —
100 p.p.m.	10	1	1,870,000	0 1–2 sec.
100 p.p.m.	10	2	330,000	0 1–2 sec.
100 p.p.m.	10	4	6,000	0 1–2 sec.
No treatment	—	0	266,000,000	—
25 p.p.m.	10	4	2,300,000	53 1–2 sec.
100 p.p.m.	10	2	10,000	0 1–2 sec.
200 p.p.m.	10	2	5,500	0 1–2 sec.
600 p.p.m.	10	2	260	0 1–2 sec.
185 p.p.m. (+milk)*	10	2	1,600	0 1–2 sec.
No treatment	—	0	93,000,000	—
200 p.p.m.	11	½	720,000	9,200 10 sec.
				0 1 min.
				0 4 min.
		6	2,200	0 10 sec.
				0 1 min.
200 p.p.m.	11·5	½	32,000,000	1,240,000 10 sec.
				0 1 min.†
				0 4 min.
200 p.p.m.	11·5	6	1,700	0 10 sec.
				0 1 min.

* 200 p.p.m. + 0·2% milk was 185 p.p.m. when used.

† Thus, even at pH 11·5, about 60 million organisms were destroyed within 1 min.

Table 15. *The destruction of bacteria in the solutions on trays is more rapid than on the surface. Trays with wet films, i.e. not dried at* 37° C.

Date	Chlorine in the solution	pH	Exposure time (min.)	Bacteria surviving: On trays	Bacteria surviving: In the solution poured off*
13. iii. 40	No treatment	—	0	260,000,000 (mean of 2 trays)	
	Tap water	7·4	½	266,000,000	11,500,000
	50 p.p.m.	8·0	½	53,000	0
	50 p.p.m.	8·0	2	500*c*	0
	50 p.p.m. + W.A.	8·5	½	2,000*c*	4,000*c*
	=39 p.p.m.	8·5	2	50*c*	0
19. iii. 40	No treatment	—	0	109,000,000 (mean of 4 trays)	
	Tap water	7·4	½	51,000,000	36,000,000
	Tap water	7·4	½	113,000,000	23,000,000
	50 p.p.m.	7·9	½	94,000	1,300
	50 p.p.m.	7·9	½	34,000	200*c*
	50 p.p.m.	7·9	2	1,300	500*c*

W.A. = 0·25% organic wetting agent added to the solution. At time of use available chlorine was 39 p.p.m. and 1 hr. later 36 p.p.m.

* Additional exposure time ⩾ 2 sec. *c* = approximately, i.e. <20 colonies on the plates.

The relationship between chlorine concentration and the rate of destruction of organisms dried on trays

The tests described in this section were done at pH 10 using the same buffer, 0·125% soda ash, in all cases. The results, using solutions of 25, 100, 200 and 400 p.p.m. chlorine, are shown in Table 16 and Charts 5 and 6.

The position of the regression lines for solutions with 25 and 200 p.p.m. chlorine are fairly dependable, but the position of the lines for 100 and 400 p.p.m. chlorine in Chart 6 is only approximate.

Increasing the concentration 8 times, from 25 to 200 p.p.m., reduced the time for 99% destruction by about 85% and for 99·9% destruction by some 80%, i.e. a solution containing 25 p.p.m. chlorine took respectively 7 and 5 times longer than one containing 200 p.p.m. at *p*H 10.

If doubling the concentration at all levels resulted in constant percentage reductions in the times for 99 and 99·9% kill, these percentage reductions would be 49 and 42% respectively. The data are probably insufficient to state whether or not this is so, but the indication is that there is a greater reduction when the concentration is increased from 100 to 200 p.p.m. than from 200 to 400 p.p.m., e.g. the figures for 99% kill at the two levels were about 60 and 30% respectively (Chart 6) and 55 and 20% (Chart 5). Weber &

Table 16. *The relationship between concentration of available chlorine at* *p*H 10 *and* 60° F.

All solutions buffered with 0·125% Soda ash.

			1	2	3	4	5	6		
Date of exp.			23. iv. 41	29. iv. 41	2. v. 41	7. v. 41*	16. v. 41	4. vi. 41		
Mean of control trays† ...			266	115	370	320	197	110		
Chlorine (p.p.m.)	*p*H	Exposure (min.)	Bacteria surviving per million						Mean log survivors per million	
25	10	½	510,000	713,000	—	—	—	—	5·781	—
(25·2–28)		2	60,000	160,000	27,000	—	—	—	4·804	—
		4	8,640	20,000	7,900	—	—	—	4·045	—
		8	—	—	110	330	—	—	2·280	—
100	10	½	17,000	103,000	9,200	—	—	—	4·402	—
(100–105)		1	—	30,000	—	5,200	4,540 5,850	—	4·097	—
		2	—	5,500	330	921	813 1,030	—	3·074	—
		4	—	—	510	27	34 28 19	—	2·216	—
									1st 3 exps.	6 exps.
200	10	½	7,140	24,300	12,300	—	5,120	33,600	4·110	4·113
(199–210)		1	240	3,220	5,950	3,820	412	3,270	3·221	3·229
		2	20	29	325	41	13	118	1·758	1·679
									3 exps.	200 p.p.m.‡
400±10	10	½	—	16,500	1,540	—	—	4,100	3·673	4·334
		1	—	1,740	1,050	—	—	346	2·934	3·599
		2	—	4	7*c*	—	—	29	0·970	2·015

* Each determination is the mean of two or three trays. † Figures in millions.

‡ Mean log of three comparative trials with 400 p.p.m. on the same dates.

Levine (7) found that doubling the concentration of chlorine water reduced the killing time by 45–50% for spores in suspension, while Rudolph (5) showed that it was necessary to quadruple the concentration of calcium hypochlorite to effect a reduction of 50% in the killing time at *p*H 10 with similar spores.

The difference in shape of the destruction curve for solutions with 25 p.p.m. chlorine compared with solutions of higher concentration cannot be attributed to differences in the buffer. A change in the shape of such curves with increasing concentrations of a disinfectant is, however, not an unusual phenomenon. We have no clear explanation as to why the solutions with 200 p.p.m. chlorine at *p*H 11 and 11·5 gave similar curves to that of 25 p.p.m. at *p*H 10. It seems clear that there are only the two types of curves, one showing a fairly constant reaction velocity from the start, the other indicating a reduction

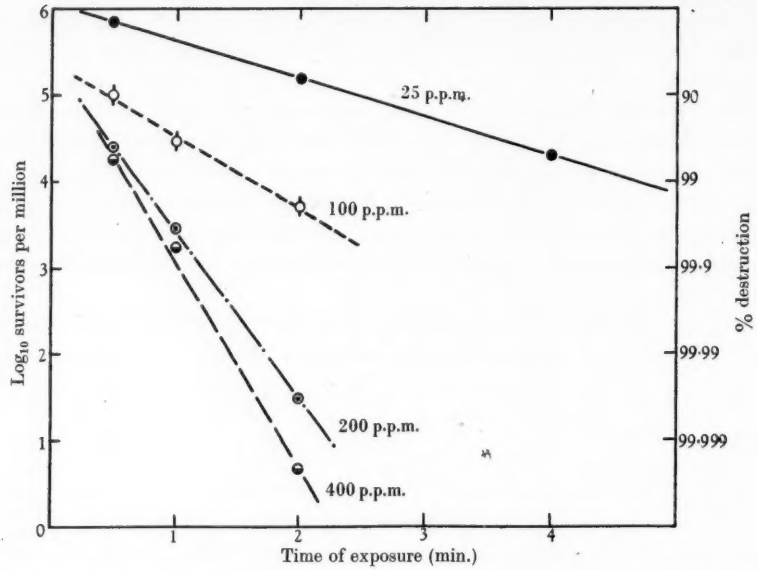


Chart 5. The relationship between concentration of available chlorine at *p*H 10 and 16° C. *Staphylococcus aureus* in a 9 % milk suspension dried on trays. All solutions buffered with 0·125 % soda ash. Points refer to results from single trays and the tests were all made on the same date, 29 April 1941. Lines fitted by method of least squares.

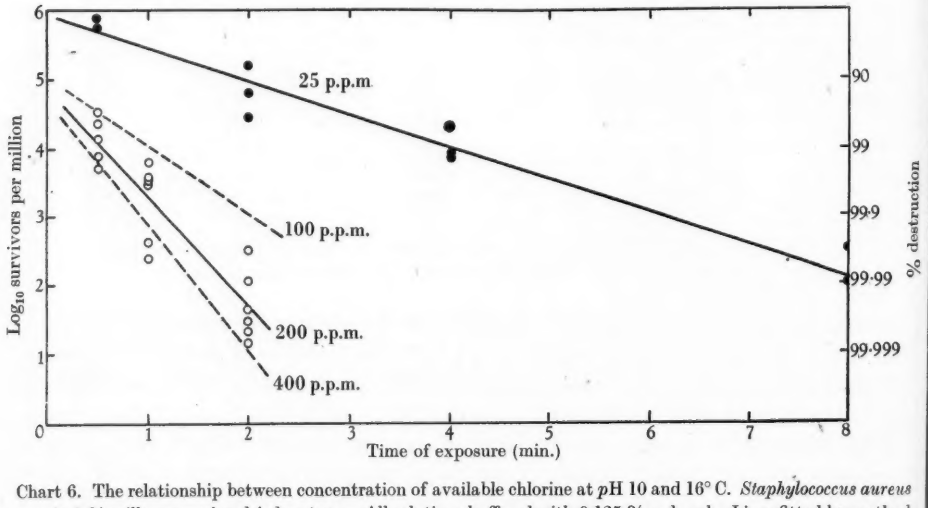


Chart 6. The relationship between concentration of available chlorine at *p*H 10 and 16° C. *Staphylococcus aureus* in 9 % milk suspension dried on trays. All solutions buffered with 0·125 % soda ash. Lines fitted by method of least squares, but points refer to tests with 25 p.p.m. and 200 p.p.m. available chlorine only. Based on replicated tests.

25 p.p.m. (3 trials): $b = -0{\cdot}48 \pm 0{\cdot}015$ 200 p.p.m. (6 trials): $b = -1{\cdot}61 \pm 0{\cdot}092$

in velocity after 90–95% of the organisms have been killed or removed. Both types of curves, however, flatten out when between 99·99 and 99·999% of the bacteria have been destroyed (Charts 1–3).

The curves of destruction for the solutions at *p*H 7 and 11·5 converge and would appear to meet at the point where they flatten out; thus if comparisons are made between these solutions at this stage (about 99·997% destruction), little difference will be found in their efficiency (Chart 2).

In an earlier experiment where agitation of the liquid on the trays was not continuous for more than 2 min., the destruction of the organisms was very slow after the first

Table 17. *Experiment designed to show that the poor penetrating power of dilute chlorine solutions in the absence of continuous agitation is not due to serious loss of germicidal power*

Solution agitated for 2 min. only.

Particulars	Chlorine p.p.m. Before	Chlorine p.p.m. After*	*p*H	Exposure time (min.)	Bacteria surviving per tray	Destroyed (%)	Removal of dried film during exposure
Freshly prepared solution (buffered with 4 ml. of 10% $NaH_2PO_4.2H_2O$ per l.)	26	—	7·08	0	250,000,000	—	Very slight in all cases, i.e. <20%
	—	—	—	0·5	16,000,000	—	
	—	22·4	—	2	2,100,000	—	
	—	21	—	8	730,000	—	
	—	—	—	12	175,000	—	
	—	21·3	—	16	180,000	—	
Freshly prepared solution (buffered with 3 ml. of 10% $NaH_2PO_4.2H_2O$ per l.)	12·2	—	7·20	0·5	132,000,000	—	
	—	9·0	—	2	12,000,000	—	
	—	7·5	—	16	900,000	—	
	—	7·1	—	24	1,400,000	—	
	—	6·9	—	32	1,200,000	—	
—	—	—	—	0	112,000,000	—	
Fresh solution	12·7	—	7·02	5	7,600,000	93·3	
Fresh solution on tray for a second period of 5 min.	12·7	—	7·02	5+5	360,000	95·3†	
Fresh solution	8·2	—	7·13	5	7,500,000	93·3	
Used solution, i.e. solution of 12·7 p.p.m. 15 min. after use for 5 min. on a tray	8·5	—	7·1	5	8,400,000	92·5	
1 ml. of the bacteria in milk suspension added direct to 230 ml. of fresh chlorine solution	8·2	—	7·18	1	0	—	
	—	—	—	5	0	—	
1 ml. of suspension added to 230 ml. of used chlorine solution	8·5	—	7·16	1	0	—	
	—	—	—	5	0	—	

* Within 2 min. of pouring off the trays.

† 95·3% of 7,600,000.

2 or 3 min. contact with the chlorine solution. Weak chlorine solutions were being used, and it was necessary to show that this reduction in germicidal velocity was not primarily because of a loss of available chlorine and/or a loss of germicidal power of the solution.

Six trays were exposed to a solution of *p*H 7 containing 12 p.p.m. available chlorine. Three of these were tested bacteriologically after 5 min. (agitated 2 min.) and three were treated for a second period of 5 min. with a fresh solution. The percentage of bacteria destroyed in the second 5 min. period was shown to be of the same order (about 95%) as in the first 5 min. period.

The concentration of chlorine after 5 min. contact with the trays had fallen to about 8·5 p.p.m. when estimated, within 2 min. of use, by both iodometric and orthotoluidine methods.

The germicidal power of the used solutions was compared with that of fresh solutions of the same chlorine concentration and found to be of the same order both for bacteria on the trays and for bacteria in suspension. As expected, the bacteria in the suspension (*c.* 100 million) were all killed in 1 min. or less. In this latter case there was little increase in the *p*H of the solution on the addition of the milk suspension as the final concentration of milk was less than 0·05%.*

From these results (Table 17) it would seem that the poor destruction obtained when agitation was not continuous cannot be caused by the loss of available chlorine or by the Mallmann effect, but is probably the result of non-agitation of the liquid and consequent slower hydration of the dried films and relative absence of available chlorine at the reacting surfaces.

The effect of the addition of organic matter to the hypochlorite solutions

Mallmann (15) and also Bryan *et al.* (16) have shown that the usual titration methods of determining the available chlorine in solutions does not reflect the germicidal power of such solutions if considerable organic matter is present.

When properly used, solutions containing chlorine should contain little or no foreign material, certainly in our experience not more than the equivalent of 0·1% milk. Several tests were made of the efficiency of a solution before and after the addition of 0·2% whole milk. The milk was added to a solution containing 200 p.p.m. chlorine, and after standing for half an hour the available chlorine and germicidal power were determined. These findings are recorded in Table 18 and do not show any significant difference in the

Table 18. *The effect of the presence of* 0·2% *whole milk on the rate of destruction by sodium hypochlorite*

Chlorine (p.p.m.)	*p*H	Buffer	Exposure (min.)	Date of experiment: 23. iv.	29. iv.	2. v.	7. v.	Mean log survivors per million
				Bacteria surviving in millions				
0	—	—	0	266	115	370	320	—
200	10·0–10·06	Soda ash, 0·125%	½*	7,140	24,300	12,300	—	4·11
			1	240	3,220	5,950	3,820	3·37
			2	20	29	325	—	1·76
200 + 0·2% Milk	10·01–10·06	—	½	8,650	24,200	12,000	—	4·13
			1	600	20,000	7,200	1,880	3·74
			2	6*c*	96	140	—	1·64
p.p.m. of chlorine before adding milk				210	200	199	204	—
p.p.m. of chlorine 30 min. after adding milk and just before use				185	168	185	184	—

germicidal power between the solution with milk and that without milk. The available chlorine content of the former had decreased about 15–30 p.p.m. before it was used. With 200 p.p.m. chlorine and only 0·2% milk there is insufficient organic matter to exhibit the phenomenon shown by Mallmann. If the milk had remained in contact for a longer period and more tests had been made, there is no doubt that a significant decrease in germicidal power would have been shown.

* The addition of small amounts of milk to weakly buffered hypochlorite solutions results in an increase of *p*H. One per cent. milk may cause an increase of 0·3–0·5 *p*H.

Increasing the thickness of dried films

A few trial tests were made to demonstrate the reduction in the efficiency of hypochlorites which would be expected to occur when the amount of organic matter per unit area was increased, i.e. when the thickness of the dried-milk film was increased.

Two methods of increasing the thickness of films were adopted: (1) by keeping the total amount of organic matter per tray the same but increasing the size of the drops of 9% milk by reducing their number from 2000 to about 30, and (2) by increasing the total amounts of organic matter through the use of whole milk instead of 9% milk, i.e. the organisms were suspended in boiled whole milk instead of 9% milk. One ml. of suspension was applied in all cases.

The approximate relative thickness of some of the films produced is shown in Table 19 and the bacteriological results in Table 20.

Table 19. *Approximate relative thickness of milk film on trays used in experiments*

Material on trays	No. of drops per tray	Mean diam. of dried drops (mm.)	Total area of dried film (dried drops) (sq.in.)	Total* organic matter (O.M.) (mg.)	O.M. per square inch of film (mg.)	O.M. ratio or relative thickness of film
9% milk (S)	2000	2	9·3	12	1·3	1
9% milk (L)	30	7	1·8	12	6·7	5·2
Whole milk (L)	30	7	1·8	120	67	52

* Milk with say 12% organic matter. (S) = small drops. (L) = large drops.

Table 20. *Increasing the thickness of dried milk films markedly reduces the efficiency of hypochlorite*

150 p.p.m. available chlorine for 1 min.

Tray no.	Material on trays	Untreated trays: Drying time (hr.)	Untreated trays: Millions: Per tray	Untreated trays: Millions: Mean	Test trays: Solutions pH	Test trays: Survivors	Mean survivors per million	Killed (%)	Film washed off (%)
1	9% milk, small drops	½	290	230	10·2	422,000	—	—	10
2		½	177		10·2	48,000?	1,000	99·9	10
3		½	124	210	8·7	580,000	—	—	10
4		½	295		8·7	650,000	3,000	99·7	10
5	30% milk, small drops	1	427	280	8·7	3,000,000*c*	—	—	25
6		1	130		8·7	2,700,000*c*	10,000	99	25
7	Whole milk, small drops	1½	530	581	10·2	10,000,000*c*	—	—	90
8		1½	633		10·2	15,000,000*c*	20,000	98	90
9	9% milk, large drops	1½	408	375	10·2	24,000,000*c*	—	—	20
10		1½	343		10·2	18,000,000*c*	60,000	94	20
11		1½	322	330	8·7	10,000,000*c*	—	—	20
12		1½	336		8·7	16,500,000	40,000	96	20
13	Whole milk, large drops	1½	562	511	10·2	120,000,000*c*	—	—	5
14		1½	460		10·2	160,000,000*c*	280,000	72	5

c = approximate only. Estimated number of colonies on crowded plates.

Control and treated trays were made in duplicate. Tests were done at *p*H 10·2, with the solution containing 0·25% soda ash, and, on a different date, at *p*H 8·7 with no added alkali or buffer. The two series are therefore not strictly comparable though very similar.

The thicker films naturally took longer to dry, and there was evidence of some growth during actual drying; this, together with the extra protection of the thicker film, probably

accounts for the larger number of organisms recovered from untreated trays having the thicker films.

Where excessive amounts of the film were not removed during the 1 min. treatment (see Table 20, last column), notably trays 1–4 and 9–14, a rough idea of the effect of the thicker films is seen. About 25 times as many bacteria were left when the individual films were increased about 12–13 times in area and consequently about 5·2 times in thickness. When whole milk replaced 9% milk the percentage killed in 1 min. exposure was only about 72 compared with more than 99·7.

The results appear to indicate that the diameter as well as the thickness of the films is a controlling factor on the rate of destruction of the bacteria trapped in the film, as, of course, is that of the tenacity of the films and their resistance to hydration and penetration by the solutions.

The amount of butter-fat present in the films must influence the results considerably. As is well known, most washed dairy utensils are covered with a more or less greasy film, and it is undoubtedly this which reduces the corrosive action of chlorine on many metals and explains why for instance aluminium teat-cups can be dipped into strong chlorine solutions (1000 p.p.m.) daily over a period of years without apparent deleterious effect. In this connexion it is worth recording that Zoller (18) found whole milk had no greater chlorine demand than skim milk.

The addition of a wetting agent to the chlorine solutions

The addition of a wetting agent to the disinfectant used on the trays would be expected to cause a marked increase in the rate of wetting, hydration and consequent removal of the dried films. This was found to be the case in the preliminary tests carried out by us using various commercial preparations of anionic surface active compounds. Some of the results are shown in Table 21.

Table 21. *Results obtained with the addition of an organic wetting agent to chlorine solutions*

Solution agitated for 2 min. only.

Chlorine in the solution	*p*H	Added wetting agent* (%)	Exposure time	Bacteria surviving on tray	Degree of wetting of film† (%)	Removal of dried film during exposure†
—	—	—	0	200,000,000	—	—
200 p.p.m.	8·52	0	10 sec.‡	3,290,000	50	Nil to slight
200 p.p.m.	8·52	0	30 sec.	760,000	50	Nil to slight
200 p.p.m.	8·52	0	2 min.	1,300*c*	50	Nil to slight
200 p.p.m.	8·52	0	5 min.	1,000*c*	50	Nil to slight
100 p.p.m.	8·32	0	30 sec.	23,000,000	50	Nil to slight
100 p.p.m.	8·32	0	2 min.	1,000,000	50	Nil to slight
100 p.p.m.	8·32	0	5 min.	75,000	50	Nil to slight
100 p.p.m.	8·16	0·03	2 min.	51,000	50	Slight
(96 p.p.m.)	8·16	0·03	5 min.	18,000	80	25%
100 p.p.m.	8·5	0·08	10 sec.‡	8,900,000	80	Nil
(88 p.p.m.)	8·5	0·08	30 sec.	4,100,000	100	Nil to slight
(88 p.p.m.)	8·5	0·08	2 min.	79,000	100	50%
(88 p.p.m.)	8·5	0·08	5 min.	1,500*c*	100	50%
Nil	8·0	0·08	5 min.§	100,000,000*c*	100	50%
100 p.p.m.	8·6	0·16	2 min.	0	100	75%
(75 p.p.m.)	8·6	0·16	5 min.	0	100	90%
Nil	8·2	0·16	5 min.	30,000,000	100	90%

* Active ingredient sodium oleyl sulphate. Percentages refer to the active ingredient.

(96 p.p.m.) = concentration of available chlorine 15 min. after use.

† Determined by inspection. *c* = approximate only.

‡ In these two cases the chlorine solution was poured off the trays after 10 sec. and the trays allowed to drain for 5 min. before adding the thiosulphate rinse. § Test on rinse from tray 2 hr. later = 30,000,000.

There is little or no published evidence that organic wetting agents used at a concentration and *p*H at which they themselves are not germicidal increase the reaction velocity of hypochlorite solutions for bacteria in suspension. Our findings confirm the fact that they can be extremely useful as an aid in the disinfection of surfaces if the films are not very tenacious.

It is our experience, however, that the artificial films used by us are much more readily hydrated and removed than some films found in practice on farm utensils and milking machines. Results of tests made on milk cans, which will be reported elsewhere, showed little improvement from the addition of a wetting agent to the hypochlorite solution used.

Trays sprayed with chlorine solutions

A few trials only have been made comparing the difference in efficiency between rinsing and spraying the infected trays.

The chlorine solutions were made up with NaOCl to contain 200 p.p.m. available chlorine and had a *p*H of about 8·5. These solutions were used on the trays as previously described and also sprayed with and without the addition of a wetting agent consisting of a mixture of sulphated higher alcohols (C_{10} to C_{18}). The concentration of active material used was about 0·12%, and this had a chlorine demand of only 5 p.p.m. in 1 hr.

The dried trays (with 9% milk suspension) were stood on end and sprayed with a medium-fine spray. The spray nozzle was held at a distance of 1 ft. and delivered the chlorine solution at the rate of 500 ml. per min., about 80% of this actually striking the tray. At any one moment the spray covered only about two-thirds of the surface, and the nozzle had therefore to be moved slightly to secure complete wetting. The spraying times given (Table 22) are thus about one-third longer than the true times. Trays were drained for 1–2 min. before testing, i.e. before adding the thiosulphate solution.

Table 22. *Comparison between a chlorine rinse and a chlorine spray. Bacteria recovered from treated trays expressed as survivors per million. Untreated or control trays had about* 200,000,000 *bacteria*

A. Solution poured on trays and agitated. 200 p.p.m. chlorine. *p*H 8·5–8·7.

Time of exposure (sec.)	Volume of solution per tray (ml.)	Wetting of surface (%)	Exp. III	Exp. V	Exp. VI
10	230	—	20,000	30,000	25,000
30	230	—	4,400	2,000	800
120	230	—	<100	<100	<100

B. Sprayed, 200 p.p.m. chlorine. *p*H 8·4, nozzle distance 1 ft.

			Exp. I		Exp. II		
5	40	15	730,000	1,200,000	900,000	—	
10	80	15	2,900	16,000	11,000	73,000	(nozzle at 2 ft.)
20	160	15	—	14,000	—	—	

C. Sprayed, 200 p.p.m. chlorine, with 0·12% wetting agent. *p*H 8·7, nozzle distance 1 ft.

			Exp. I		Exp. II		Wetting agent alone
1½	12	95	30,000	—	—	—	0·12%
5	40	100	19*	86	6,000	6,000	170,000
							170,000*
10	80	100	32	134	61	80	40,000

* Tray drained for 10 min. after spraying, i.e. before adding thiosulphate solution.

The results which are shown in Table 22 are sufficient to show that spraying for 10 sec. is slightly less efficient than rinsing the trays for 1–2 min. On the other hand, the addition of a wetting agent increases considerably the efficiency of the spray if it is capable of removing much of the film. Under the conditions described satisfactory results were obtained with a 10 sec. spray using about 100 ml. of solution per square foot.

The wetting agent used was germicidal* at *p*H 8·7, but as shown in section C of Table 22, a drainage period of 10 min. before adding the thiosulphate solution to the tray did not apparently reduce the count further, and the effect is therefore chiefly that of wetting and detergency. The thiosulphate rinses from trays where the wetting agent had been used showed a reduction of only about 15% in the number of viable organisms after standing on the bench for 45 min.

Temperature coefficient

All our tests have been made with solutions at about 16° C., but a word might be said about recent findings in respect of the temperature coefficient of chlorine. This coefficient is apparently about 2 for a change of 10° C., but recent publications differ as to whether the coefficient increases or decreases with increasing temperature. Estimates of the coefficient based on the destruction of *Bact. coli* in the presence of 0·25% organic nitrogen showed a decrease with increase in temperature (17). Rudolph (5), working with the spores of *Bacillus metiens* at *p*H 10, found the coefficient to be 1·86 for the temperature interval 20–30° C. and 2·40 for the interval 30–40° C., while Weber & Levine (7) found the coefficient to be 2·1–2·4 in both cases. The corresponding values (with chlorine in the presence of organic nitrogen) found by Ames & Smith (17) using *Bact. coli* were 2 and 1·5.

It is of interest to note that the estimates of the temperature coefficient made by Rudolph for 'calcium hypochlorite' correspond very closely to those found by Zoller (18) in 1923 when he was determining the effect of temperature on the rate at which milk absorbed available chlorine. Zoller found that 100 ml. of milk absorbed 100 p.p.m. available chlorine in 22 min. at 20° C., 10·5 min. at 30° C. and 4·5 min. at 40° C.

We do not know to what extent unstable chloramines are formed in the milk films during the exposure of the trays to solutions of hypochlorites, nor what part, if any, they play in the destruction of the bacteria. That caseinogen may be chlorinated has been shown by Wright (19, 20), who also showed that the product was more stable at *p*H 7 than at a higher *p*H.

The destruction of bacterial spores in suspension

Until the investigation of Levine and his co-workers, Charlton & Levine (25), Rudolph (5), Weber (6) and Weber & Levine (7), there had been little or no systematic study of the effect of the *p*H, temperature and concentration of chlorine solutions on the rate of destruction of bacterial spores.

The effect of the *p*H of solutions, prepared from calcium hypochlorite (B–K) and from chlorine gas, on the destruction of the spores of *Bacillus metiens* is shown in Table 23. The germicidal time equivalents, shown at the bottom of the table, appear to indicate that solutions at *p*H 7 are 34–200 times as efficient as solutions at *p*H 10. The great difference in the germicidal velocity at *p*H 10 reported for solutions prepared from calcium hypochlorite and from chlorine gas is not evident at *p*H 6 or 7 and requires confirmation.

* When the organisms were added direct to an 0·2% solution their numbers were reduced by 50% in 2 min. and 90% in 10 min.

The approximate concentration coefficients of solutions at the same *p*H were for calcium hypochlorite 1·4 (Rudolph (5)) and for chlorine water 1·9 (Weber & Levine (7)).

The time, reported by a number of workers, for the destruction of the spores of various organisms is recorded in Table 24. Few of the figures shown are comparable because of

Table 23. *The effect of p*H *on the time for the destruction of* 99% *of the spores of* Bacillus metiens *in aqueous suspension*

	Temp. 20° C.		
Author	Rudolph (1942)	Weber (1942) and Weber & Levine (1944)	
Source of available chlorine	Calcium hypochlorite	Chlorine gas	Chlorine gas
p.p.m. of available chlorine ...	25	25	48
*p*H	(min.)	(min.)	(min.)
5	—	2·1	1·2
6	2·5	2·3	—
7	3·5	3·0	1·4
8	5	7·6	—
9	19·5	58	—
9·35	35·3	—	—
10	121	570	291
12·86	465	—	—
	Germicidal time equivalents (from above)		
7	1	1	1
9	5·6	19	—
10	34·6	190	208

Table 24. *The destruction of bacterial spores in suspension by available chlorine from hypochlorites or chlorine water. Representative data from the literature**

Reference	Spores	*p*H	p.p.m. available chlorine	Time for 99–99·9% destruction	Temp. ° F.
Tilley & Chapin (23)	*B. anthracis*	6	6	15 min.	70
Prucha (24)	Thermophil	6 ?	50	1–5 min.	120
Scales & Kemp (27)	*B. subtilis*	6	50	5 min.	70
Rudolph (5)	*B. metiens*	6	25	2·5 min.	70
Rudolph (5)	*B. metiens*	8	25	5 min.	70
Charlton & Levine (25)	*B. metiens*	7·3	1000	<20 sec.	77
Weber & Levine (7)	*B. metiens*	7·0	50	1·4 min.	68
Myers (26)	*B. cereus*	7·5	100	1 min.	140
Charlton & Levine (25)	*B. metiens*	8·2	20	5 min.	77
Myers (26)	*B. cereus*	8–9	500	12 min.	77
Prucha (24)	Thermophil	9–10	300	20 min.	120
Tilley & Chapin (23)	*B. anthracis*	9	50	45 min.	70
Rudolph (5)	*B. metiens*	9·35	25	35 min.	70
Rudolph (5)	*B. metiens*	10	25	120 min.	70
Weber & Levine (7)	*B. metiens*	10	188	94 min.	68
Myers (26)	*B. cereus*	11·4	100	22 min.	140 (same as 1% NaOH)
Scales & Kemp (27)	*B. subtilis*	9·5	255	>5 min.	70
Charlton & Levine (25)	*B. metiens*	10·4	100	70 min.	77
Charlton & Levine (25)	*B. metiens*	11·3	1000	64 min.	77
Costigan (13)	*Mycobacterium tuberculosis*	8	50	2·5 min.	122
				0·5 min.	140
Costigan (13)	,,	9	200	1·0 min.	122

* See also Cousins & Wolf (21).

the difference in the *p*H and concentration of the solutions, and also because of some differences in the experimental methods not shown in the table. Adopting a concentration coefficient of 2 it appears from the above table that at *p*H 6 *B. metiens* is more susceptible to available chlorine than *B. anthracis*, and that *B. subtilis* is more resistant than either.

The actual difference in susceptibility, if any, could only be determined under the same experimental methods using a number of strains.

From estimates based on the work of Rudolph (5) we should expect spores in aqueous suspension to be destroyed in about 30 sec. by 200 p.p.m. available chlorine at 180–200° F. if the *p*H was 10–10·5.

At *p*H 10 and 70° F. Rudolph reported 99% of the spores killed in 31 min. by a solution containing 500 p.p.m. available chlorine.

Discussion

It is a well-known fact that bacteria in aqueous suspension, when exposed to hypochlorite solutions, are very much more rapidly killed when these solutions are acid or neutral than when they are alkaline. It has been shown, however, that in a solution at *p*H 11 containing 200 p.p.m. available chlorine, few bacteria, washed off a contaminated surface, will survive more than a few seconds, unless more resistant than the *Staphylococcus aureus* strain used in our investigations.

In the disinfection of surfaces, the bacteria remaining on the surface are often of much greater importance than those rinsed off, and it has been demonstrated that in the case of bacteria in thin whole-milk films, where the films are not completely removed during exposure to hypochlorite solutions, solutions at *p*H 11 are as rapid as or more rapid in their disinfectant action than solutions at *p*H 7, and more rapid than solutions above *p*H 11.

We have suggested, as a possible explanation for this phenomenon, the strong local buffering action of the partially hydrated films, together with the more rapid removal of the films by the alkaline solutions. No satisfactory explanation was found for the poor germicidal power of solutions above *p*H 11.

Since this paper was completed, Cousins & Wolf (21) have produced confirmation that the vegetative cells of bacteria in dried-milk films are destroyed less rapidly by neutral than by alkaline solutions of hypochlorites, but they point out that this does not apply to the spores of bacteria. They postulate the formation of chloramines on the surface of vegetative cells (but not spores), and that these chloramines, which are less markedly affected by the *p*H of solutions than are hypochlorites, are responsible for the destruction of the bacteria in the films.

There is little doubt that chloramines of varying stability are formed when chlorine reacts with proteins or amino acids of milk (Rupp (22), Wright (19, 20) and others). We should agree that chloramines formed by the interaction of chlorine with nitrogenous compounds in the film are very probably responsible for some of the germicidal action.

Weber (6) and Weber & Levine (7) showed that chloramine from chlorine and ammonia was twice as rapid in action at *p*H 7 and 8 than at *p*H 9 and 10. We must therefore assume that the chloramines or chloroproteins formed as postulated by Cousins & Wolf have a different *p*H optimum, or else that their films exposed to the solutions at *p*H 10–11 were removed very much more rapidly than the films exposed to solutions at *p*H 7 or 8.

Wright's (19, 20) work, which showed that chlorinated caseinogen, when certain concentrations of available chlorine and protein are allowed to react, has an optimum stability near *p*H 7 and is more unstable in solutions between *p*H 7 and 12·5, may be worth examining further.

The above remarks would presumably apply also to the films containing spores and are therefore of little apparent use in elucidating the problem. With the data at present available it seems that the postulation of Cousins & Wolf must be accepted tentatively. There is no doubt that the chemical as well as the physical nature of the films must influence the results greatly, and there is a need for a much fuller investigation.

Summary

Tinned trays were artificially infected with *Staphylococcus aureus* in diluted milk and the dried surfaces disinfected by rinsing with sodium hypochlorite solutions.

Under the conditions described, which we believe approximate those on many dairy utensils which have been imperfectly cleaned, the *p*H of hypochlorite solutions was found not to be a very important factor in determining their germicidal efficiency. At *p*H 10 and 11, the solutions were equal to or slightly more efficient germicides than solutions at *p*H 7.

In the destruction of 99% of the organisms on the metal trays, a solution at *p*H 10, containing 200 p.p.m. available chlorine was about 7 times as rapid as one with 25 p.p.m. available chlorine.

Spraying the trays for 10–20 sec. was found to be less efficient than rinsing them for 1–2 min., though the addition of an organic wetting agent to the solution resulted in the spray being more effective than the rinse, because of the rapid removal of the artificial milk film.

The addition of 0·2% whole milk to a hypochlorite solution containing 200 p.p.m. available chlorine did not significantly reduce its efficiency within 30 min.

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348. SOME MODIFICATIONS OF THE SCHIBSTED FAT ALDEHYDE TEST

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The application of the Schibsted fat aldehyde test for the routine examination of butter and butterfat is facilitated by the following modifications:

(1) Storage of the reagent at cool temperatures.
(2) Use of sodium metabisulphite instead of sulphur dioxide.
(3) Preparation of the standard colour at a lower *p*H.

1. Storage of the reagent at cool temperatures

Precautions for preserving the qualities of the rosaniline reagent are mentioned by Schibsted (1), p. 206), who states: 'As oxygen slowly oxidizes the sulphur dioxide, it is advisable to exclude oxygen from the bottle holding the reagent or to divide the freshly made reagent into several small bottles completely filled and well stoppered.' In practice, however, it has been found that in addition to the precautions mentioned by Schibsted, a low temperature for storage is essential.

The following experiment illustrates the necessity for this precaution:

Four small brown-glass bottles were filled completely with rosaniline reagent and corked. Two more bottles were filled partially. Two of the full bottles were placed in a refrigerator at 4–5° C., the remaining two full bottles and the partially filled bottles being placed in a cupboard at room temperature.

A sample of olive oil was tested with another portion of the rosaniline reagent and the aldehyde value was found to be 1·2. The sample of oil was then placed in the refrigerator.

After 17 days the olive oil was examined again with the three portions of stored rosaniline reagent. The following results were obtained:

	Aldehyde value
Reagent in full bottles stored at 4–5° C.	1·2
Reagent in full bottles stored at room temperature	0·6
Reagent in partially filled bottles stored at room temperature	0·05

The reagent had thus not deteriorated during 17 days at 4–5° C., whereas a marked deterioration had taken place at room temperature.

2. Use of sodium metabisulphite instead of SO_2

It was thought that the sodium salt would be less sensitive to oxidation than the sulphurous acid.

Two solutions of rosaniline hydrochloride were prepared:

Reagent 1. Usual method with SO_2 according to Schibsted.

Reagent 2. Use of 1·42 g. of sodium metabisulphite in place of 0·85 g. SO_2.

The two reagents were compared on a pharmaceutical preparation of cod-liver oil. Both of the freshly prepared reagents gave an aldehyde value of 19.

The reagents were then bottled in small brown narrow-mouth bottles, corked and stored in a refrigerator at 4–5° C. for comparison after 10 days' storage, and thereafter at weekly intervals. The reagents were also stored in the same type of bottles but not completely filled. The results of this trial were:

1A. SO_2 used as reagent, stored in full bottles at 4–5° C.

2A. Metabisulphite used as reagent, stored in full bottles at 4–5° C.

1B. SO_2 used as reagent, stored in incompletely filled bottles at 4–5° C.

2B. Metabisulphite used as reagent, stored in incompletely filled bottles at 4–5° C.

	After 10 days	After 17 days	After 24 days
1A	19·0	19·0	19·0
2A	19·0	19·0	19·0
1B	5·5	2·5	Nil
2B	19·0	10·0	2·7

It is seen, therefore, that the sodium metabisulphite solution gives the same results as the sulphur dioxide solution when fresh, and is not so easily oxidized as the solution of sulphurous acid. In partially filled bottles, however, it also deteriorates at 4–5° C. The reagent is more easily prepared with the sodium metabisulphite since titration with iodine is eliminated.

It is suggested that as an alternative to storing the prepared reagent at 4–5° C., the rosaniline hydrochloride in alcohol be stored at room temperature. When the reagent is required, 100 ml. of the alcoholic solution are transferred to a 200 ml. measuring flask, an aqueous solution of 0·284 g. of sodium metabisulphite solution is added, and the volume of the reagent is made up to the mark with distilled water. Under this procedure the reagent is always freshly prepared.

3. Preparation of the standard colour with cresol red

It is probable that Schibsted based the tint of the standard colour on determinations with lard and other fats and oils with low content of yellow colour. A buffer solution of *p*H 8·3 gives with cresol red a colour in close agreement with that of a sample of lard, but with butterfat there is a noticeable discrepancy in tint.

The composition of the buffer solution suggested by Schibsted corresponds to a formula designed by Palitsch (2), and it has been found that when the *p*H of the buffer solution is reduced to 7·8 the cresol red gives the desired tint. The formula for the buffer solution at *p*H 7·8 is:

Sodium borate	3·822 g.
Boric acid	9·923 g.
Sodium chloride	2·925 g.

to be dissolved in water and made up to 1 l. The buffer solution is then used to dissolve the cresol red according to Schibsted's directions.

The author is indebted to Dr F. H. McDowall for helpful advice.

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349. A SIMPLE METHOD OF CONDUCTING THE PHOSPHATASE TEST

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The value of the phosphatase reaction for controlling the heat treatment of milk is unquestioned, but the accepted method of estimating phosphatase activity, namely, the colorimetric method of Kay using the phenol reagent of Folin, has three disadvantages:

(1) The number of estimations that can be performed in a given time is limited.

(2) Errors in matching the tints may occur.

(3) Samples of milk may be accidentally contaminated with traces of phenol, and this will lead to too high a Lovibond value.

The test to be described has none of these disadvantages. This test depends on the liberation of phenolphthalein from its phosphoric ester.

The colour of the phenolphthalein can be detected in the presence of the constituents of the milk, and this eliminates the deproteinization and filtration necessary in the Kay test. The investigator has to decide whether the diluted milk is pink or not—thus eliminating the comparator required for the Kay test. Moreover, phenolphthalein, unlike phenol, is never likely to find its way accidentally into milk.

Even if this method should be slightly less accurate than the Kay method, which can only be decided by further experiment, it should serve as a very useful 'preliminary screening' test when many samples have to be tested.

Phenolphthalein phosphoric acid, as a substrate for phosphatase, was introduced by King(1), and was used by Bray & King(2) to investigate bacterial phosphatase. It is worthy of note that these authors found several of the bacteria commonly found in milk to be fairly potent producers of phosphatase.

Technique

We have used in our test the same dilution of the milk as is used in the Kay method, namely, 0·5 c.c. of milk made up to 10 c.c. with soluble barbitone as a buffer.

We have not made any attempt to determine what might be more favourable conditions for the hydrolysis of the phenolphthalein phosphate in milk because we decided to use the simplest technique and compare the results of this method with those obtained by the Kay test.

The buffer substrate solution is 11·55 g. of soluble barbitone per 1000 c.c. water into which is dissolved, with boiling, 0·1 g. of the calcium salt of phenolphthalein phosphate.

If the solution contains any free phenolphthalein, which can be detected by the addition of ammonia to the sample, it should not be used.

The milk test is done by adding 10 c.c. of the phenolphthalein phosphate barbitone solution to 0·5 c.c. of milk, a few drops of chloroform being finally added and the tubes incubated for 24 hr. at 37° C. At the end of that time, 0·25 c.c. of a 1 in 10 dilution of liquor ammoniae fortis is added. The development of a pink tint indicates the presence of active phosphatase in the milk; no colour indicates complete destruction of the enzyme, and consequently adequate heating of the milk.

The test depends on the presence or absence of colour, not on variations in the intensity of a colour as happens in the Kay test.

Under the conditions described above, sufficient phenolphthalein is liberated by a raw milk, after 5 min. incubation at 37° C., to give a readily detectable pink flush.

The following was done to assess the possible value and experimental error of the test when applied to samples from a pasteurizing plant. With the kindly co-operation of Mr C. H. Chalmers, Chief Milk Inspector, Department of Health for Scotland, three dairies engaged in processing milk were asked to submit samples to us without giving any information as to whether these had, or had not, been processed. The Kay phosphatase tests on these samples were performed by the staffs of the dairies. The results of their phosphatase tests and the degree of heat treatment were not disclosed to us until the series was completed. For our own guidance we did the Kay test on those samples which gave no pink tint or a pink tint that was markedly less intense than that given by a raw milk.

Table 1 indicates the heat treatment applied to these specimens and the results obtained by the two Kay tests and the new phenolphthalein test on all the samples received from Dairy A.

Table 1. *Synopsis of results, Dairy A*

Temp. to which specimens exposed ° F.	Time of exposure (min.) 5: Reference number of specimen	Kay test at dairy	Phenolphthalein phosphate test	Kay test at laboratory	10: Reference number of specimen	Kay test at dairy	Phenolphthalein phosphate test	Kay test at laboratory	20: Reference number of specimen	Kay test at dairy	Phenolphthalein phosphate test	Kay test at laboratory	30: Reference number of specimen	Kay test at dairy	Phenolphthalein phosphate test	Kay test at laboratory
140	1	X	X	—	2	X	X	—	3	X	X	—	4	X	X	—
	25	X	X	—	26	X	X	—	27	X	X	—	28	X	X	—
145	5	X	X	—	6	X	X	—	7	X	X	—	8	**P**	**F**	**2·8**
	29	X	X	—	30	X	X	—	31	X	X	—	32	**F**	**?**	**2·3**
150	9	X	X	—	10	**F**	**?**	**2·3**	11	**F**	**P**	**2·3**	12	Y	Y	Y
	33	X	X	—	34	X	X	X	35	**P**	**F**	**2·8**	36	**P**	**F**	**2·7**
155	13	Y	Y	Y	14	Y	Y	Y	15	Y	Y	Y	16	Y	Y	Y
	37	Y	Y	Y	38	Y	Y	Y	39	Y	Y	Y	40	Y	Y	Y
		0			1				5				10			
160	17	X	X	X	18	**P**	**?**	**2·3+**	19	Y	Y	Y	20	Y	Y	Y
	41	**P**	**F**	**6**	42	Y	Y	Y	43	Y	Y	Y	44	Y	Y	Y
165	21	X	X	X	22	Y	Y	Y	23	Y	Y	Y	24	Y	Y	Y
	45	**F**	**P**	**<2·3**	46	Y	Y	Y	47	Y	Y	Y	48	Y	Y	Y

X = failed—no discrepant tests. Y = passed—no discrepant tests.
F = failed and **P** = passed—findings discrepant.
Figures = Lovibond readings Kay test; 2·3 or **<2·3** = pass, >2·3 = failed, **2·3+** = just over 2·3.

It will be noted from the table that the discrepancies in the holder-pasteurized samples occurred at the figures critical for adequate pasteurization—30 min. at 145° F. and 10, 20 and 30 min. at 150° F.

It was arranged that before sending the samples by post to our laboratory some chloroform would be added to stop bacterial growth, but by an oversight this was not done in specimens numbered 1–36. It is, therefore, possible that the bacterial content of

samples 8, 35 and 36 was much higher in the sample examined by us than in the one examined at the dairy, and the slightly higher phosphatase value found in our laboratory might be due to the activity of bacterial phosphatase.

The phenolphthalein gave a definite, but not strong, pink tint in these cases and the Lovibond value was slightly, but to our eye definitely, over the 2·3 limit, and for these reasons the milk was classed as 'Failed' by us.

Table 2

No. of sample	Kay test at dairy	Phenolphthalein test	Kay test at laboratory
8	Passed	Failed	Failed
35	Passed	Failed	Failed
36	Passed	Failed	Failed
41	Passed	Failed	Failed
18	Passed	?	Just over 2·3
10	Failed	?	Passed
32	Failed	?	Passed
11	Failed	Passed	Passed
45	Failed (2·3–6)	Passed	Passed (<2·3)

Table 3

		Dairy B				Dairy C			
Temp. ° F.	Time of exposure (min.)	Sample no.	Kay tests made at dairy	Phenol-phthalein tests	Kay tests made at laboratory	Sample no.	Kay tests made at laboratory	Phenol-phthalein tests	Kay tests made at laboratory
140	5	49	>6	F	—	71	>2·3	F	—
	10	50	>6	F	—	72	>2·3	F	—
	20	51	>6	F	—	73	>2·3	F	—
	30	52	>6	F	—	74	>2·3	F	—
145	5	53	>6	F	—	75	<2·3	P	<2·3
	10	54	>6	F	—	76	2·3+	P	<2·3
	20	55	>6	F	5·5	77	2·3+	P	<2·3
	30	56	>6	F	>2·3	78	<2·3	P	<2·3
150	5	57	>6	F	9	79	2·3+	P	<2·3
	10	58	<2·3	?	2·3	80	2·3+	P	<2·3
	20	59	<2·3	P	<2·3	81	2·3+	P	<2·3
	30	60	1·5	P	<2·3				
155	5	61	>6	F	—*				
	10	62	2·3–6	F	—*				
	20	63	2·3–6	F	—*				
	30	64	1·5	F	—*				
160	0	65	>6	F	—				
	1	66	<2·3	F	—				
	5	67	2·3	P	<2·3				
	10		No specimen						
165	0	68	1·5	?	2·3				
	1	69	1·5	P	<2·3				
	5	70	1·5	P	<2·3				
	10		No specimen						

F = failed to pass test; P = passed test; ? = doubtful; — = not done; * = sour.

The disagreement in samples 10, 11 and 32 probably represents difference of opinion on whether the blue colour was darker or lighter than the standard colour on the Lovibond disk. It is in such cases that the phenolphthalein test proves useful because, as already mentioned, the decision is whether the milk shows a pink tint or does not.

When all the discrepancies—nine in number—are collected together as in Table 2, it is found that when the Kay test and the phenolphthalein phosphate tests are compared

on the same samples, the agreement between the two tests is good. The difference in the Kay tests, as mentioned before, may be due to either bacterial growth in transit or the personal factor in comparing the blue colours.

The results in the case of samples from dairies B and C are shown in Table 3.

In the case of dairy B the colorimetric readings are given as reported to us, and 2·3+ means that the reading is just over 2·3.

Specimens 61–64 inclusive, marked *, were sour and actually clotted on receipt. For that reason we do not propose to make any conclusions from the results on these samples. The only discrepancy is sample no. 66, which we failed and the dairy passed. In this case we did not do the Kay test because the phenolphthalein test showed a definite pink tint and the milk was 'failed' by us.

In the samples from dairy C attention is directed to samples 75–78, which appear to have been heat-treated before delivery at the dairy. Samples 79, 80 and 81, which were passed by phenolphthalein test and our Kay test but failed at the dairy, probably represent different interpretations of the colour matching in the Kay test.

In addition to these samples submitted by dairies A, B and C co-operating in the work, other samples were examined.

(i) *A series of samples from an* H.T.S.T. *plant*

This is a particularly valuable series of observations as the system of heating and cooling was of high efficiency, and accurate comparison between the five samples could be made:

Ref. no.	Temp. ° F.	Time of exposure (sec.)	Kay test	Phenol-phthálein test
82	150	15	>2·3	F
83	154	15	>2·3	F
84	158	15	>2·3	F
85	160	15	2·3	?
86	162	15	<2·3	P

(ii) *Samples from eleven different pasteurizing plants of various kinds*

Ref. no.	Kay test at dairy	Phenolphthalein test	Kay test at laboratory
87	2·3	P	2·3
88	1·8	P	<2·3
89	1·8	P	<2·3
90	2·1	P	<2·3
91	2·1	?	2·3
92	3	?	2·3+
93	2·1	P	<2·3
94	2·1	P	<2·3
95	1·5	P	<2·3
96	1·5	P	<2·3
97	3	F	>2·3

(iii) *Routine tests on samples from an* H.T.S.T. *plant*

Of these nos. 98–104 all passed both the Kay test and the phenolphthalein test.

CONCLUSIONS

1. The findings show that the procedure suggested as an alternative to the Kay and Graham method of performing the phosphatase test on milk is worthy of further investigation.

2. Using a very simple technique, results are obtained which are remarkably concordant with the accepted method.

3. As a 'screening procedure' it could be used, even in this simple fashion, to save unnecessary labour.

4. Further investigation with a view to determining the optimum conditions for performing the test and ascertaining its experimental error is proceeding.

5. The test can be adapted, using a range of dilutions, roughly to assess the phosphatase content of blood plasma. Both acid and alkaline blood phosphatase reaction can be shown if the appropriate acid and alkaline substrates are used.

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350. THE EFFECT OF SODIUM CHLORIDE ON THE *p*H VALUES OF MILK AND OF BUTTER SERUM

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I. Introduction

Mohr & Ritterhoff (1) found that the addition of 10% of common salt to milk lowered the *p*H value as measured by the hydrogen and glass electrodes by 0·29 and 0·31 unit respectively. They did not discuss the cause of this change. In salted butter churned from ripened cream (*p*H 4·65) they found the *p*H of the serum 0·3 unit lower than in unsalted butter from the same cream. Hunziker, Cordes & Nissen (2) found the *p*H of cream, salted butter and buttermilk to be practically the same between *p*H 6·50 and 6·70. Above *p*H 6·70 the butter had a higher *p*H than the buttermilk. These butters were made from neutralized cream, but the alkali used is not specified. McDowall, Smith & McDowell (3) stated that on the whole for most factories in New Zealand, the *p*H of the cream gave a reasonably accurate indication of the *p*H of the resultant butter. They discussed the effect of loss of carbon dioxide during churning, and of the alkalinity of the wash water, and apparently assumed that apart from such factors the *p*H of the butter should be the same as that of the cream.

Townley & Gould (4) found that when cream was neutralized with sodium carbonate the *p*H of the butter was 0·15 above that of the cream, but when lime or magnesia was used as neutralizer the *p*H of the butter was 0·37 above that of the cream. Differences at *p*H 6·0 varied little from those at *p*H 7·0 to 7·5.

Wiley & Newman (5) found that at about *p*H 7·5 the *p*H of the butter was on the average 0·06 unit above that of the cream, while at *p*H 6·5 the *p*H of the butter was on the average 0·14 below that of the cream. There was considerable variation in individual churnings. The authors mentioned that both the salting and the dilution of the serum during churning would affect the *p*H. White (6) found that at *p*H 6·84 the *p*H of salted butter serum averaged 0·19 unit below that of the cream. At *p*H 6·16 the difference was 0·16, and at *p*H 5·55 the difference was 0·08.

II. Experimental

(*a*) *General*

In an investigation of the effects of sodium chloride and of hydrogen-ion concentration on the oxidation of butterfat at 40° C., use was made of an artificial inorganic butter serum containing 0·2% sodium citrate, 0·2% potassium chloride, saturated with monocalcium phosphate, and adjusted with hydrochloric acid to *p*H 6·98. When this solution was made 1·6*M* with respect to sodium chloride the *p*H measured by the glass electrode dropped to 6·11. It was essential in this work to be able to vary the sodium chloride content without altering the *p*H, and the cause of this effect was therefore investigated.

In addition, it seemed that information was needed on two points: why the *p*H of milk decreased on adding sodium chloride, and why a similar difference was not normally observed between the *p*H of cream and the *p*H of butter churned from it.

The error of the glass electrode in acid solutions or water-alcohol mixtures, caused by its functioning as a water electrode, is given by Dole (7) as $\Delta E = RT/F \ln a_{H_2O}$, where a_{H_2O} is the activity of the water. This formula was confirmed by Amis & Gabbard (8) for neutral solutions of magnesium sulphate. Applying this formula to a 1·7*M* (10%) sodium chloride solution and using figures in *Int. Crit. Tables* for the vapour pressure of sodium chloride solutions as a measure of the activity of the water, the calculated error is −0·02 *p*H. The other type of error to which the glass electrode is subject, caused by its functioning as a sodium electrode in alkaline solutions, was found by Dole, Roberts & Holley (9) to be negligible below *p*H 8·0. Checking the change in *p*H on adding sodium chloride with another glass electrode, with a quinhydrone electrode, and qualitatively with indicators, confirmed that the change could not be explained as a salt error of the electrode. Change in liquid junction potential cannot be invoked as an explanation, since the change in e.m.f. to be expected from a 1·7*M* concentration of a monovalent electrolyte such as sodium chloride is much less than the observed change. Experimental evidence presented later (Table 3) also indicates that the change in e.m.f. is not due to change in the liquid-junction potential.

Several lots of sodium chloride, including some that had been recrystallized, and a heated commercial salt of initially slightly alkaline reaction, gave closely similar results in these experiments. The A.R. quality sodium chloride, mainly used when prepared as a saturated solution in CO_2-free water, showed no acidity, 100 ml. giving the same intensity of colour with phenolphthalein on adding 2 drops of 0·1*N* caustic soda as did 100 ml. of CO_2-free distilled water.

(*b*) *The effect of added sodium chloride on the p*H *values of various salt solutions*

Sodium chloride was added to solutions of salts containing ions present in milk serum, and the resulting changes in *p*H are recorded in Table 1. Sodium citrate and sodium

Table 1. *The effect of sodium chloride concentration on the p*H *of several salt solutions*

Salt solution	*p*H when concentration of sodium chloride is								
	Zero	0·01*M*	0·02*M*	0·05*M*	0·10*M*	0·20*M*	1·0*M*	1·7*M*	2·0*M*
Sodium citrate 0·007*M* + HCl	6·92	6·82	6·75	6·70	6·59	6·42	6·00	5·85	5·76
Di-sodium phosphate 0·33*M* + HCl	7·82	—	—	—	—	—	—	7·22	—
Di-sodium phosphate 0·033*M* + HCl	8·10	—	—	—	—	—	—	7·20	—
Di-sodium phosphate 0·0033*M* + HCl	8·17	—	—	—	—	—	—	7·12	—
Calcium chloride 0·018*M* + NaOH	6·80	—	—	—	—	—	—	5·95	—
Sodium bicarbonate 0·024*M*	8·30	—	—	—	—	—	—	7·60	—
Calcium lactate 0·015*M*	6·42	—	—	—	—	—	—	6·03	—

phosphate solutions showed marked changes in *p*H on addition of sodium chloride. Lowering the concentration of sodium phosphate increased this change in *p*H. The *p*H of calcium chloride, sodium carbonate and calcium lactate solutions also dropped appreciably on adding sodium chloride.

The change in *p*H on adding sodium chloride must be due either to a change in ionic

activities resulting from the change in ionic strength of the solution, or to specific ionic association. It is possible to distinguish between the effects, as Acree and his co-workers (10) have done with sodium chloride in buffer solutions, by comparing the change in *p*H when the buffer salt instead of sodium chloride is used to increase the strength.

The ionic strengths of molal solutions of sodium chloride, sodium citrate and sodium phosphate at *p*H 6·5 are approximately 1·0, 6·0 and 2·0 ($\mu = \frac{1}{2}\Sigma CZ^2$, where C = concentration and Z = charge on each ion). The figures in Table 2 show that when an acidified solution of sodium citrate of an ionic strength of 2·0 is diluted to one-tenth and the ionic strength again made equal to 2·0 with sodium chloride, there is a drop in *p*H of 0·45 unit. The smaller change in *p*H when sodium phosphate was treated in the same way is also recorded in Table 2. Such measurements should ideally be made in a system without

Table 2. *The effect on the pH of sodium citrate and sodium phosphate solutions when the ionic strength is increased by adding sodium chloride, sodium citrate or sodium phosphate*

Ionic strength contributed by			Total ionic strength	
Sodium citrate (μ)	Sodium phosphate (μ)	Sodium chloride (μ)	(μ)	*p*H
0·2	—	—	0·2	7·05
2·0	—	—	2·0	6·70
0·2	—	1·8	2·0	6·25
—	0·2	—	0·2	6·76
—	2·0	—	2·0	6·28
—	0·2	1·8	2·0	5·98

liquid junctions. If the summing up of Bates, Hamer, Manov & Acree (11), that in normal *p*H determinations (in solutions of 0·05–0·1 *M* concentration) liquid-junction potentials may account for an error of 0·01–0·05 *p*H be accepted, then it would be reasonable to conclude that there is evidence of specific ionic association. Later, however, reference was made to the work of Sager, Keegan & Acree (12) who found that in acid-salt solutions up to 2 *M* concentration and containing bivalent ions, liquid junction potential errors may reach 0·3–0·4 *p*H. Although in the present case the ionic strengths at the liquid junction were equal, the difference being in ionic type and mobility, it would seem unwise to draw any definite conclusion from this evidence as to the degree of ionic association between the sodium and citrate or phosphate ions.

Hastings & McLean (13) showed that there is strong association of calcium and citrate in the Ca citrate ion. The effect of small amounts of calcium chloride on the change in *p*H of sodium citrate and sodium phosphate solutions on adding sodium chloride was studied. The results are shown in Table 3.

The addition of 0·2% of calcium chloride to an acidified sodium citrate solution reduced the *p*H more than the addition of 10% of sodium chloride, and at the same time inhibited the change in *p*H with addition of sodium chloride. The addition of the same concentration of calcium chloride to sodium phosphate reduced only slightly the *p*H change on adding sodium chloride.

The addition of fat-globule membrane substance, obtained by churning washed cream, or of soya-bean lecithin, to sodium citrate solutions did not affect the change in *p*H with sodium chloride.

Table 3. *The effect of calcium chloride on the change in pH of sodium citrate and sodium phosphate solutions on adding sodium chloride*

Initial solution	Calcium chloride added (%)	pH	On making 1·7 *M* with respect to sodium chloride	
			pH	Change in pH
Sodium citrate 0·2% + HCl	None	6·89	5·75	−1·14
Sodium citrate 0·2% + HCl	0·01	6·80	5·75	−1·05
Sodium citrate 0·2% + HCl	0·02	6·71	5·72	−0·99
Sodium citrate 0·2% + HCl	0·04	6·55	5·66	−0·89
Sodium citrate 0·2% + HCl	0·06	6·22	5·62	−0·60
Sodium citrate 0·2% + HCl	0·10	5·72	5·52	−0·20
Sodium citrate 0·2% + HCl	0·20	5·44	5·42	−0·02
Sodium phosphate 0·2% + HCl	None	6·28	5·40	−0·88
Sodium phosphate 0·2% + HCl	0·20	6·02	5·42	−0·60

(c) *The effect of added sodium chloride on the pH values of various salt solutions containing alkaline precipitates*

A solution containing 0·2% sodium citrate, 0·2% disodium phosphate, and 0·2% calcium chloride was adjusted to *p*H 6·2. Caustic soda was then added to bring the *p*H to 7·98. A gelatinous precipitate formed. When this was filtered off and the filtrate made 1·7 *M* with respect to sodium chloride, the *p*H dropped to 7·22. When the same concentration of sodium chloride was added to the solution in the presence of the precipitate, the *p*H rose to 8·05. The same results were obtained when lactic acid was added to the solution before adding caustic soda. Dilution 1 : 1 of the salted filtrate with 1·7 *M* sodium chloride caused the *p*H to drop by 0·04. Similar dilution of the salted solution plus precipitate caused the *p*H to rise by 0·40 unit.

The increase in *p*H in the presence of the precipitate on adding sodium chloride and also on diluting with sodium chloride solution is presumably due to the solubility of the alkaline precipitate in sodium chloride. Holt, La Mer & Chown (14) have shown that the solubility of dicalcium phosphate, and to a much greater extent that of tricalcium phosphate, is increased by sodium chloride. Another possible explanation is release from the precipitate by sodium chloride of adsorbed hydroxyl ions. That the effect is due mainly to calcium phosphate, and to a lesser extent to calcium citrate, is suggested by the results, given in Table 4, of an experiment in which the salts were precipitated separately.

Table 4. *The alkaline effect of precipitates of calcium citrate and calcium phosphate in solutions of sodium chloride*

Salts in solution	*p*H on precipitating with alkali	*p*H on diluting 20% and making 1·7 *M* with NaCl	
		In presence of precipitate	Filtrate
Sodium citrate and calcium chloride	8·15	7·70	7·35
Sodium phosphate and calcium chloride	8·18	8·47	6·74

In numerous experiments artificial inorganic butter serums of varying composition were neutralized with caustic soda, sodium bicarbonate or sodium carbonate. In all cases the presence of the precipitate reduced or reversed the change in *p*H on adding sodium chloride and diluting 20% (butter serum is normally diluted to about this extent during

washing and moisture adjustment). Evidence was sought whether adjustment to higher *p*H (8·0 instead of 6·5) gave a more alkaline precipitate, since this appeared a possible explanation of the observation by Wiley & Newman(5) that the *p*H of alkaline butters was above, while that of slightly acid butters was below, the *p*H of the cream from which they were churned. Several tests showed rather the opposite tendency, and typical figures including some for acid milk whey are given in Table 5. It was found, however, that change in the ratio of calcium to phosphorus markedly altered the alkaline effect of the precipitate (Table 5).

Table 5. *The effect of p*H *of precipitation and of* Ca : P *ratio on the alkaline effect of the precipitate in sodium chloride solution*

	*p*H when	
	pptn sl. acid	pptn alkaline
Neutralized solution	6·53	8·30
Neutralized solution diluted 20% and made 1·7 *M* with NaCl	6·72	8·21
Filtrate diluted 20% and made 1·7 *M* with NaCl	5·84	7·17
Acid whey neutralized	6·67	7·97
Neutralized acid whey diluted 20% and made 1·7 *M* with NaCl	6·72	7·87
Filtrate diluted 20% and made 1·7 *M* with NaCl	6·11	—
	Ca : P ratio 2·2 : 1	Ca : P ratio 1·06 : 1
Neutralized solution	8·18	8·47
Neutralized solution diluted 20% and made 1·7 *M* with NaCl	8·47	7·63
Filtrate diluted 20% and made 1·7 *M* with NaCl	6·74	7·67

Boiling the precipitated solution, which caused a considerable drop in *p*H, presumably by conversion of precipitated dicalcium phosphate to tricalcium phosphate, greatly reduced the alkaline effect when sodium chloride was added in the presence of the precipitate, as is shown in Table 6. This may be taken to support the conception that addition of sodium chloride causes release of adsorbed hydroxyl ions, rather than solution of the precipitate itself, but insufficient is known of the chemical nature of the precipitate to arrive at a definite conclusion. Heating to 90° C. for 1 min., which is roughly equivalent to flash pasteurization, slightly reduced the alkaline effect of the precipitate.

Table 6. *The effect of boiling the precipitated solution on the alkaline effect of the precipitate in sodium chloride solution*

	*p*H	
	Unboiled solution	Boiled solution
Neutralized solution	8·24	7·12
Neutralized solution made 1·7 *M* with sodium chloride	8·13	6·27 (after 3 days, 6·28)
Filtrate made 1·7 *M* with sodium chloride	7·24	6·20

(*d*) *The effect of sodium chloride on the p*H *of milk*

Twelve samples of milk were made 1·7 *M* with respect to sodium chloride, and the change in *p*H was measured with the glass electrode. The change varied from −0·36 to −0·49, and averaged −0·42 unit.

Milk was coagulated with rennet and the whey filtered off. The whey was boiled and again filtered to precipitate and remove as far as possible any residual colloidal calcium

phosphate. The decreases in *p*H of the original milk and of the whey on making 1·7 *M* with sodium chloride were 0·40 and 0·60 respectively.

When acid whey was precipitated with alkali the addition of sodium chloride in the presence of the precipitate at *p*H 6·67 caused a rise in *p*H, while the addition of sodium chloride to the filtrate caused a drop in *p*H. The figures are given in Table 5.

(e) The concentration of precipitated calcium in butter during churning

It appeared possible that calcium salts precipitated in the cream by neutralizing and pasteurizing might be concentrated in the butter serum during churning by flotation with the fat globules in the froth, by simple embedding through impact with the soft fat globules, or adsorption of colloidal precipitate on to the fat globules.

Several lots of commercial butter were churned from cream at various *p*H levels without washing of the butter granules, and a butter sample was taken after the first working, before water was added, so that no dilution of the butter serum took place. Butter and buttermilk from the churnings were analysed for calcium, phosphorus, total solids-not-fat and moisture content, and the concentrations of calcium, phosphorus, and solids-not-fat per 100 g. of water are recorded in Table 7. The generally low level of

Table 7. *Increase of concentration of solids-not-fat, calcium and phosphorus in the aqueous phase of butter compared with the aqueous phase of buttermilk*

		g. per 100 g. water					
		Solids-not-fat		Calcium		Phosphorus	
Churning	*p*H of buttermilk	Butter	Buttermilk	Butter	Buttermilk	Butter	Buttermilk
1	7·50	8·72	5·53	0·161	0·073	0·135	0·063
2	7·50	8·15	5·62	0·160	0·073	0·135	0·064
3	7·50	7·60	4·74	0·161	0·063	0·133	0·057
4	7·35	—	—	0·154	0·071	—	—
5	7·35	—	—	0·168	0·068	—	—
6	7·35	—	—	0·147	0·058	—	—
7	6·14	7·23	5·73	0·108	0·081	0·120	0·065
8	6·14	6·25	5·12	0·099	0·073	0·107	0·059

the figures compared with those in milk is due to dilution of the cream during processing, which included pasteurization with direct steam.

Considerable concentration of solids-not-fat occurs in the butter during churning, particularly in the alkaline butters. At *p*H 6·14 the increase of calcium is approximately that to be expected if all the additional solids-not-fat are tetracalcium caseinate. At *p*H 7·50 the increase in calcium is double that calculated on the same basis. The increase in phosphorus is also considerably greater than can be accounted for as casein and phospholipide (using the figures for phospholipide in butter and buttermilk given by Mohr, Brockmann & Müller(15)). There is thus evidence that in the churning of alkaline creams there occurs in the butter a concentration, relative to the water, of precipitated calcium phosphate and of other milk solids-not-fat.

III. Discussion

From the study of the change in *p*H on addition of sodium chloride to solutions of salts containing ions present in milk serum, it is clear that the change in *p*H on adding sodium chloride to milk will largely depend on the proportion of the various ions, particularly

calcium, citrate and phosphate ions, in the milk serum. Incidentally, the proportion of these ions affects the heat stability of milk, so that it is conceivable that the heat stability of milk may be related to the change in *p*H on adding sodium chloride. Little definite information on the ionic composition of milk serum is available. From what is known, the average change in the *p*H of milk, 0·42, is less than might be expected. It is possible that in fresh milk, as in neutralized milk-salt solutions or neutralized whey, the colloidal calcium phosphate reduces the drop in *p*H when sodium chloride is added. This is suggested by the greater drop in *p*H in boiled and filtered rennet whey than in the milk from which it was prepared. The buffering effect of milk proteins at *p*H 6·6 is slight, and removal of the proteins should not in that way affect the *p*H change. It cannot be assumed, however, that sodium chloride does not affect the equilibrium between the colloidal calcium caseinate phase and the aqueous phase.

From the experimental results recorded it is clear that two opposing tendencies will mainly control the relation between the *p*H of cream and the *p*H of the serum of salted butter made from it. The first of these, tending to lower the *p*H of the butter, occurs solely in the aqueous phase and is due to change in ionic activities and perhaps to specific ionic associations on addition of sodium chloride. The second, tending to increase the *p*H, depends on interaction of the sodium chloride solution with the dispersed solid phase of precipitated alkaline calcium salts. One or both of these tendencies are affected by the various factors listed below. The relation between the *p*H of cream and the *p*H of the salted butter made from it will therefore depend on the following factors as well as on the alkalinity of the wash water and loss of CO_2 during churning:

(1) The initial salt composition of the milk.
(2) Destruction of citrate during souring.
(3) Alteration of base-binding capacity of the protein by bacterial action.
(4) The alkali used for neutralization.
(5) The *p*H to which the cream is adjusted.
(6) The extent of water dilution during processing particularly after neutralizing and pasteurizing.
(7) The pasteurization time and temperature.
(8) The amount of salt added.

IV. Summary

The addition of 10% of sodium chloride to milk caused an average drop in *p*H of 0·42 unit. Different workers have found very variable relations between the *p*H of cream and the *p*H of salted butter churned from it. When 10% of sodium chloride is added to 0·2% solutions of sodium citrate or sodium phosphate there is a drop in *p*H of the order of 1 unit. When an alkaline solid phase is present as in the serum of neutralized cream and butter, and in milk, this change in *p*H may be diminished or reversed, possibly by increased solubility of the alkaline precipitate in sodium chloride solution. Factors are listed which will cause the *p*H of salted butter to differ from that of the cream from which it was made. In the churning of alkaline creams there occurs in the butter an increase in the proportion to the serum water of calcium phosphate and of other solids-not-fat.

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351. THE ESTIMATION OF COPPER AND IRON IN CREAM, BUTTER AND DRY BUTTERFAT

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Introduction

The pro-oxidative effect of traces of copper and iron in fats is well established, and measures for control of quality of fats usually include an estimation of the degree of contamination with these metals. The oxidation of the butterfat of cream and butter is accelerated by minute quantities of copper and iron, and dry butterfat is even more susceptible. The method of estimation of copper and iron in these products has been investigated by the author in connexion with the work on dry butterfat production (1), and the results of the investigation are now presented.

Wet-ashing methods. The accurate estimation of copper and iron in dairy products has generally involved a preliminary dry-ashing (2) or wet-ashing (3) of the samples. Recent work indicates that any dry-ashing method for the estimation of either copper (4) or iron (5) in biological materials and foods will lead to erroneous results, especially with a product such as butter which contains 82% of fat. Wet-ashing methods are more satisfactory, and a number of such methods have been described.

Williams (3) wet-ashed either butter or cream by digestion in beakers first with nitric acid alone and then with sulphuric acid after the withdrawal of fat. He determined both copper and iron in the acid digest. For copper estimation in butter, Moir & Andrews (6) and Epple & Horrall (7) adopted Williams's wet-ashing procedure but digested in Kjeldahl flasks, thus saving time and material. For iron estimation in a number of foods, including milk, Roberts *et al.* (8) digested with nitric and sulphuric acids in micro-digestion flasks.

The present author has found the most suitable vessel for use in the wet digestion of cream, butter, and butterfat to be a micro-Kjeldahl digestion flask, which prevents copper or iron contamination from the air and yields up only traces of the metals to the digesting liquid. Accurate methods of estimation of the metals in the acid digest have been developed, and only small quantities (5 or 10 g.) of the sample are required for analysis.

Simplified methods for cream and butter. The wet-ashing procedure is time-consuming and is unsuitable for routine estimations. The various alternative methods which have been suggested for the rapid estimation of iron and copper in cream and butter (9, 10, 11) are suitable only for approximate determinations of relatively large quantities of the metals. The nearest approach to a simple yet reasonably accurate method of estimation is the 'filtration method' of Moir & Andrews (6).

Although copper and iron may exist in cream and butter as minute particles from abrasion of metal parts of machinery, it is generally assumed that the metals are first dissolved by the milk or cream from an exposed surface and are subsequently bound in unionized metal protein complexes (12).

Estimation of iron. Iron is presumably taken into solution in milk or cream in the ferrous form, but rapid oxidation apparently follows (13) with immediate formation of

a complex protein compound. Thus it was found that ortho-phenanthroline (a colorimetric reagent for ferrous iron) gave no colour when added shortly after mixing a small quantity of ferrous salt with cream, nor was any of the iron (ferrous or ferric) recovered in the filtrate after precipitation of the proteins with trichloracetic acid. The protein complexes apparently are very similar to those formed by the non-haemin or 'inorganic' iron of blood, tissues, foods, etc. (13, 14, 15). This non-haemin iron is usually estimated in the filtrate after precipitation of the proteins with trichloracetic acid. An essential preliminary to the method, however, is the complete extraction of the iron from its protein complex, e.g. by boiling with 5*N* hydrochloric acid (16), by heating with pyrophosphate (14), or by the action of reducing agents (13, 17). Moir & Andrews (6) devised a similar technique for the estimation of iron in butter. They heated the sample overnight at 37° C. with sodium hydrosulphite as a reducing agent before precipitating the proteins, drawing off the fat by suction, and filtering the remaining liquid.

In a number of trials of the estimation of iron by methods very similar to that of Moir & Andrews, the author found that the extraction of the iron from the protein complex by heating with hydrochloric acid, with pyrophosphate, or with sodium hydrosulphite, was unsatisfactory. In comparison with the iron content obtained by the wet-ashing method the results were often low and erratic.

Thioglycollic acid (17, 18) proved a more satisfactory reducing agent. The addition of 5 drops of the acid to butter or cream at 50–100° C. rapidly brought the iron into solution as shown by the colour developed with ortho-phenanthroline. The problem was further simplified by the suggestion of Kitzes *et al.* (19) that the main difficulty in the estimation of iron in blood plasma was the co-precipitation of iron with the proteins and the incomplete removal of this iron by washing. They found that by heating the blood plasma at 90–100° C. a gel was formed, and after cooling the addition of trichloracetic acid resulted in a light curdy precipitate, the very nature of which facilitated the removal of any residual iron. Applying these findings to the present work it was found that by heating butter or cream in a boiling water-bath with thioglycollic acid, with subsequent cooling and precipitation of proteins by trichloracetic acid, much more consistent recoveries of iron in the filtrate were possible than with previous methods. A similar method for the determination of iron in enriched bread has recently appeared (20).

Estimation of copper. It is generally recognized that copper is much less firmly held in the protein complex than is iron. Thus Tompsett (21) found that the addition of trichloracetic acid was sufficient to bring into solution all the copper in blood. Moir & Andrews (6), in their estimation of copper in butter, added a few drops of hydrochloric acid and of 3% hydrogen peroxide to the samples before precipitation of the proteins and removal of the fat.

The author finds that the addition of hydrochloric acid and trichloracetic acid is sufficient to bring into solution the copper present in cream and butter, but otherwise the method finally adopted is very similar to that of Moir & Andrews. The estimation of copper on one portion of the filtrate for iron determination or on the same filtrate after estimating the iron by the colour of the ferrous-ortho-phenanthroline complex was attempted, but, owing probably to the presence of the reducing agent (thioglycollic acid), the recovery of copper was low. The attempt was therefore abandoned and iron and copper were estimated on separate samples.

Simplified method for dry butterfat. In the estimation of copper and iron in cream and

butter as described above, it is first necessary to liberate the metals from the protein complex. As dry butterfat contains only a trace of curd, most or all of the original copper and iron of the butter has been separated off in the serum. The fat itself during the short time it is in contact with metallic surfaces during the manufacturing process is unlikely to dissolve any appreciable quantity of the metals, and any metallic contamination of the butterfat is probably the result of abrasion in the plant itself. It was found that after filtering melted butterfat through a Whatman no. 50 filter-paper most of the copper and iron remained on the paper as shown below:

	Iron p.p.m.		Copper p.p.m.	
Sample	Before filtration	After filtration	Before filtration	After filtration
Butterfat (1)	0·12	0·05	0·10	0·01
Butterfat (2)	0·52	0·07	0·05	0·01

The metals thus appear to be present as minute particles of copper and iron or possibly, after the plant has been standing idle, as copper and iron oxides. As a preliminary step in a simplified method of estimation of the metals an acid extraction of the butterfat was attempted, and it was found that iron was recovered quantitatively by shaking the melted butterfat with dilute hydrochloric acid. Although copper does not dissolve readily in hydrochloric acid without the addition of an oxidizing agent the micro-quantities present in dry butterfat were recovered quite satisfactorily in the acid alone. The dilute acid solution and the fat were separated in the centrifuge and the fat was drawn off by suction. The acid extract was slightly turbid but filtered readily after the addition of trichloracetic acid and sodium tungstate. Copper and iron were estimated on portions of the same filtrate.

Experimental

Colorimetric reagents for iron and copper

Iron. As colorimetric reagents for iron, either potassium thiocyanate or ortho-phenanthroline are suitable, depending on the quantity of iron to be estimated. Although the thiocyanate method is often adversely criticized (22, 23) it is still widely used (8, 24), and provided the acidity of the sample and of the standard are the same and the colours are compared reasonably soon after the addition of thiocyanate the results appear to be trustworthy. Where the concentration of iron is very low, e.g. 0·15 p.p.m. or less as in butterfat, the method has one great advantage in that the intensity of the colour is greatly increased by the extraction with iso-amyl alcohol.

The ortho-phenanthroline reagent under the conditions recommended by Bandemer & Schaible (25) offers certain advantages over the thiocyanate method for larger quantities (0·005 mg. and higher) of iron. As in the estimation of iron in cream and butter (see above), thioglycollic acid proved the most suitable reducing agent. The standard solution of iron was prepared by diluting a solution of ferrous ammonium sulphate (oxidized with permanganate in the presence of sulphuric acid) with distilled water (iron-free), so that 1 ml. contained 0·01 mg. of iron.

Copper. The sodium diethyl-dithio-carbamate reagent for copper is very sensitive (26), and provided precautions are taken to prevent interference from iron, has been found very satisfactory in the estimations described in this paper. The reagent is added as a 0·1% solution in 50% ammonium citrate at *p*H 8·5 as recommended by Clare *et al.* (27). The addition of as much as 5 ml. of this reagent to 10 ml. of solution is desirable, since with

smaller quantities of the reagent fading of the yellow copper-carbamate salt in iso-amyl acetate may take place. Precipitation of phosphate is prevented by the addition of ammonium citrate to the solution, and a high concentration of this salt also has a 'salting-out' effect on the copper-carbamate compound (4) which is readily extracted with iso-amyl acetate. Ammonia is also added in excess, since a high concentration of ammonium and citrate ions prevents interference from ferric iron (28).

Concentration of the copper-carbamate salt is best carried out by extraction with iso-amyl acetate which is definitely superior to iso-amyl alcohol, in that it clears rapidly after shaking with the solution containing the copper salt (29). The addition of the carbamate reagents and the iso-amyl acetate immediately after the ammonia is an advantage as the heat of neutralization ensures a more complete extraction of the copper-carbamate salt.

The standard solution of copper was prepared from copper sulphate and contained 0·01 mg. of copper per ml.

A colorimeter was used for both copper and iron estimation. A photoelectric absorptiometer would no doubt be more convenient in practice, but such an instrument was not available.

Purification of reagents

Blank estimations are necessary in the determination of both copper and iron. If all glassware, after thorough cleansing, is washed with hydrochloric acid (concentrated acid diluted 1 : 1) and rinsed with distilled water (copper and iron-free), if filter-papers (no. 50 Whatman are preferred to no. 42) are acid-washed just before use, and if some of the reagents are purified, the copper and iron in the 'blank' sample is usually very low.

Laboratory-distilled water if not free from copper and iron must be glass-distilled.

Nitric acid for wet-ashing is redistilled.

Trichloracetic acid is redistilled and is then made up to a 20% solution.

Ammonium citrate solution (50%) is freed from copper by the addition of the carbamate reagent and extraction with iso-amyl acetate.

Potassium thiocyanate solution (50%) is freed from iron by the addition of a few drops of nitric acid and extraction with iso-amyl alcohol. Iso-amyl alcohol and iso-amyl acetate are obtained by fractional distillation of crude amyl alcohol and amyl acetate respectively, and further purification is unnecessary.

Preparation of the samples

A representative sample of butter or dry butterfat is placed in an acid-washed glass jar or bottle with a suitable cover or stopper. The butter or butterfat sample is melted at approximately 40° C., well mixed, and the approximate weight required for analysis is pipetted out. The cream sample, contained in an acid-washed glass bottle with glass or rubber stopper, is heated to 40° C. and well shaken, and the approximate weight required for analysis is pipetted out.

Detailed procedure of the wet-ashing method

Ten grams of butter or dry butterfat or 5 g. of cream (note (1)) are pipetted into a micro-Kjeldahl flask together with 1 ml. (for butters) or 2 ml. (for butterfats) of water (note (2)). Nitric acid (4 ml.) is added and the flask is placed in a boiling water-bath for 15–20 min. with frequent shaking (note (3)). After cooling for a short time the fat is extracted with three successive quantities of petrol ether, the petrol ether layer being

drawn off each time by suction. The flask is then placed on a micro-Kjeldahl digestion stand with fume tube, and the contents are digested over a micro-flame until the volume is about 0·5 ml. Concentrated sulphuric acid (0·5 ml., A.R. quality) is added and the heating is continued with further addition of a few drops of nitric acid if necessary until the acid solution is clear and nearly colourless when the nitric acid has been driven off. To complete the digestion and to destroy nitrosyl sulphuric acid, etc., two successive amounts of 0·3 ml. of perhydrol are added, and the mixture is heated to the appearance of white fumes each time.

For copper estimation the acid digest is transferred to a test-tube with 5–7 ml. of water, and 1 ml. of 50% ammonium citrate solution and 2·5 ml. of ammonia (sp.gr. 0·88) are added. The carbamate reagent (5·0 ml.) and iso-amyl acetate (3 ml.) are added at once and the test-tube is closed with an acid-washed rubber stopper and shaken vigorously. After standing a short time the colour of the clear acetate layer is compared in the colorimeter with standards prepared from standard copper solution with the addition of 0·5 ml. of sulphuric acid, 5–7 ml. of water and the same quantities of citrate and other reagents as added to the samples.

For iron estimation with potassium thiocyanate the acid digest in the micro-Kjeldahl flask is diluted nearly to the neck of the flask with water (25–30 ml.) and several drops of potassium permanganate (approximately $N/50$) are added (note (4)). Following the addition of 3 ml. of 50% potassium thiocyanate (note (5)) and 5 ml. of iso-amyl alcohol, the flask is closed with an acid-washed rubber stopper and shaken vigorously, and the colour of the alcohol layer is compared in a colorimeter with standards prepared from standard iron solution, 0·4 ml. of sulphuric acid (note (6)), 25–30 ml. of water and the same quantities of thiocyanate and iso-amyl alcohol as added to the samples.

For iron estimation with the ortho-phenanthroline reagent the acid digest is transferred to a 10 ml. graduated test-tube with 5–6 ml. of water, and several drops of $N/50$ potassium permanganate are added (note (7)). One drop of *p*-nitro-phenol is added to the solution and the *p*H is adjusted by the addition of ammonia (sp.gr. 0·88) from a burette until a yellow colour appears, and then by dilute hydrochloric acid, drop by drop, till the yellow colour just disappears. An aqueous 0·1% solution of ortho-phenanthroline (1 ml.) and 2 drops of thioglycollic acid are added, and then 1 ml. of 25% sodium citrate solution and water to bring the volume up to 10 ml. The contents of the tube are mixed by shaking and compared in the colorimeter with standards prepared from standard iron solution with the addition of 0·4 ml. of concentrated sulphuric acid, the same quantities of reagents as were added to the samples, and water to bring the volume up to 10 ml.

Notes on the wet-ashing method. (1) The quantity of cream may be increased to 10 g., in which case twice the stated quantities of nitric and sulphuric acids must be added. The acid extract, after diluting to 10 ml., can then be divided for copper and for iron estimation. This dilution of the acid extract and determination of both copper and iron may also be carried out with a 10 g. butter sample if the content of the copper and iron is known to be high. When the copper content of dry butterfat is very low it is advisable to take twice the usual quantity—20 g. instead of 10 g.—and to digest in Pyrex test-tubes ($7 \times 1\frac{1}{2}$ in.) which are of larger capacity than the micro-Kjeldahl flasks.

(2) Undiluted nitric acid is too drastic in its action on butterfat. Addition of water is not necessary with cream.

(3) This period of heating is sufficient to dissolve copper and iron not in solution.

Longer heating results in production of fatty compounds which are insoluble in petrol ether and are difficult to digest.

(4) The addition of permanganate solution ensures the removal of traces of hydrogen peroxide. Otherwise bleaching of the colour occurs in the iso-amyl alcohol extract.

(5) This high concentration of thiocyanate is necessary to ensure maximum colour development (30).

(6) The acidity of the standards will then be the same as that of the samples, presuming that 0·1 ml. of the original acid has been lost during digestion.

(7) Bleaching of the ferrous-ortho-phenanthroline colour may also occur with traces of hydrogen peroxide (see note (4)).

Detailed procedure of the simplified method for cream and butter

(*a*) *Estimation of iron*

Cream (5 g.) or butter (10 g.) is pipetted into a Babcock cream bottle (note (1)). Water (2 ml.), 5 drops of concentrated hydrochloric acid (note (2)) and 5 drops of thioglycollic acid are added, and the mixture is heated in a boiling water-bath for 5 min. with frequent shaking. After cooling to 50–60° C., 20% trichloracetic acid (1 ml. for butters and 2 ml. for creams) is added, and the mixture is shaken and returned to the water-bath at 50–60° C. for 10–20 min. The Babcock bottle containing a butter sample is centrifuged to separate the fat which is drawn off by suction. Sodium tungstate (5 drops of a 10% solution) (note (3)) is then added to both cream or butter sample bottles and the mixture is shaken and cooled, preferably in the refrigerator, for at least 1 hr. The mixture is filtered through no. 50 Whatman filter-papers (7 cm. size for butters, 9 cm. size for creams) into test-tubes graduated to contain 11 ml. The Babcock bottle and filter-paper are washed twice with 2 ml. amounts of 5% trichloracetic acid. After adding 1 ml. of 0·1% aqueous ortho-phenanthroline to the filtrate the excess acid is neutralized with 4–6 drops of ammonia (sp.gr. 0·88), and the *p*H is finally adjusted with 1 ml. of 25% sodium citrate. The volume is made up to 11 ml. with water and the colour compared in a colorimeter with standards prepared from standard iron solution, 5 drops of hydrochloric acid, 2 drops of thioglycollic acid, 2 ml. (for butters) or 3 ml. (for creams) of 20% trichloracetic acid, the same quantities of reagents as added to the filtrate, and water to 11 ml.

Notes on the method. (1) The Babcock cream bottle (B.S.S. no. 755) is very suitable for this estimation. The opening in the neck is wide enough for the introduction of the sample and reagents but narrow enough to prevent metallic contamination from the air. Also the Babcock centrifuge, which is available at butter grading stores, dairy factories, etc., is quite suitable for the separation of the fat (in butter) from the liquid layer.

(2) The addition of 5 drops of hydrochloric acid ensures that any iron present in the metallic state is dissolved.

(3) The tungstic acid formed precipitates further protein material and ensures a clear filtrate (6). It is advisable to omit this reagent from the 'blank' estimation, as tungstate in the filtrate interferes with the ferrous-ortho-phenanthroline reaction (31). With cream or butter samples, however, the tungstate is completely precipitated with the proteins.

(*b*) *Estimation of copper*

Cream (5 g.) or butter (10 g.) is pipetted into a Babcock cream bottle. Water (2 ml.) and concentrated hydrochloric acid (1 ml.) are added, and the sample is heated in a water-bath at 50° C. for 10 min. with occasional shaking. After the addition of 20% trichloracetic acid (1 ml. for butters, 2 ml. for cream), the sample is heated at 50° C. for a further 15–20 min. The Babcock bottle containing a butter sample is centrifuged to separate the fat which is drawn off by suction. Sodium tungstate (5 drops of a 10% solution) is then added to both cream or butter sample bottles and the mixture is shaken and cooled, preferably in the refrigerator, for at least 1 hr. The mixture is filtered through a Whatman no. 50 filter-paper (7 cm. size for butters, 9 cm. size for creams) into test-tubes and the Babcock bottle and filter-paper are washed twice with 2 ml. amounts of 5% trichloracetic acid. To the filtrate is added 1 drop of 3% hydrogen peroxide (to oxidize ferrous iron to ferric iron) 1 ml. of 50% ammonium citrate, 2 ml. of ammonia (sp.gr. 0·88), 5 ml. of carbamate reagent and 3 ml. of iso-amyl acetate, and the test-tube is closed with an acid-washed rubber cork and shaken vigorously. Comparison in the colorimeter is made with standards prepared from standard copper solution, 1 ml. of hydrochloric acid, 2 or 3 ml. of 20% trichloracetic acid and the same quantities of citrate and the other reagents as added to the filtrate.

Detailed procedure of the simplified method for dry butterfat

The melted butterfat (30 g.) is pipetted into a Babcock cream bottle with the addition of 4 ml. of water and 2 ml. of concentrated hydrochloric acid. The bottle is heated in a water-bath at 50° C. for 10 min. with frequent shaking, and then 1 ml. of 20% trichloracetic acid is added and the bottle is shaken and returned to the water-bath for a further 10 min. After centrifuging and drawing off the fat, 5 drops of 10% sodium tungstate are added, and the solution is cooled in the refrigerator to ensure that any remaining fat solidifies. The acid solution is then filtered through a no. 50 Whatman filter-paper into a test-tube graduated to contain 12 ml., and the bottle and filter paper are washed with 4 ml. of 5% trichloracetic acid. The volume is made up to 12 ml. with water, and, after mixing, 8 ml. are pipetted into another test-tube for copper estimation.

The remaining 4 ml. (equivalent to 10 g. of butterfat) is made up to 15 ml. with water for iron estimation, and 1 drop of 3% hydrogen peroxide and 1 drop of nitric acid (note (1)) are added. After the addition of 1·5 ml. of 50% potassium thiocyanate solution and 5 ml. of iso-amyl alcohol the test-tube is closed with an acid-washed rubber stopper and is shaken vigorously. After standing a short time the colour of the clear iso-amyl alcohol layer (note (2)) is compared in the colorimeter with standards prepared from standard iron solution, 0·7 ml. of concentrated hydrochloric acid, 0·7 ml. of 20% trichloracetic acid, water to 15 ml. and the same quantities of peroxide and other reagents as added to the filtrate.

For estimation of copper on the other portion of the filtrate (equivalent to 20 g. of butterfat) 1 drop of 3% hydrogen peroxide, 1 ml. of 50% ammonium citrate, 2 ml. of ammonia (sp.gr. 0·88) and 5 ml. of 0·1% carbamate solution are added and the copper-carbamate salt is at once extracted with 3 ml. of iso-amyl acetate. The colour is compared in the colorimeter with standards prepared from standard copper solution, 1·3 ml. of concentrated hydrochloric acid, 1·3 ml. of 20% hydrochloric acid, water to approximately 8 ml., and the same quantities of citrate and other reagents as added to the samples.

Notes on the method. (1) To convert ferrous to ferric iron Peters & French (23) suggest that hydrogen peroxide in concentrations not over 0·0064 *M* is the most satisfactory oxidant. The addition of 1 drop of nitric acid maintains oxidizing conditions (30).

(2) Should the iso-amyl alcohol layer not be clear after 15–20 min. (during which time the samples should be kept in subdued light), the addition of 1 drop of absolute alcohol with gentle shaking is effective in removing any cloudiness.

Results

A comparison of the iron contents of cream and butter samples by the wet-ashing method and the simplified method is shown in Table 1. It will be seen that though there is not always a full recovery in the simplified method—due probably to retention of iron in the protein precipitate—the results give a reasonable approximation of the quantity of iron present.

Table 1. *A comparison of results for the iron content of cream and butter samples by the wet-ashing method and by the simplified method*

	Iron p.p.m.	
Sample	Wet-ashing method	Simplified method
Cream (A)	3·85	3·7
Cream (B)	1·5	1·3
Cream (C)	1·1	1·2
Creamery butter (P14)	1·5	1·3
Creamery butter (W14)	0·9	0·9
Whey butter (K)	5·1	4·5
Whey butter (D)	5·8	5·2
Whey butter (P)	9·1	8·6
Whey butter (F)	11·6	9·9
Whey butter (J)	2·6	2·6

Results by the simplified method for copper estimation in cream and butter agree well with figures from the wet-ashing method (Table 2) and the shorter method appears quite reliable for the routine estimation of this metal.

A comparison of the iron and copper contents of dry butterfat samples by the wet-ashing method and the simplified method is shown in Tables 3 and 4. As a perfectly uniform distribution of the metals in butterfat samples is most unlikely, the results indicate that the metals may be estimated with considerable accuracy by the latter method.

Table 2. *A comparison of results for the copper content of cream and butter samples by the wet-ashing method and by the simplified method*

	Copper p.p.m.	
Sample	Wet-ashing method	Simplified method
Whey butter (W1)	0·30	0·25
Whey butter (W3)	0·32	0·25
Whey butter (B)	0·64	0·63
Whey butter (C)	0·44	0·38
Whey butter (D)	0·52	0·46
Creamery butter (K18)	0·12	0·13
Creamery butter (K19)	0·08	0·13
Creamery butter (T25)	0·14	0·11
Cream (1)	0·18	0·12
Cream (2)	0·42	0·40
Cream (3)	0·18	0·20
Cream (4)	4·1	4·4

Table 3. *A comparison of results for the iron content of dry butterfat samples by the wet-ashing method and by the simplified method*

Sample	Iron p.p.m.	
	Wet-ashing method	Simplified method
(K)	0·60	0·71
(S)	0·38	0·27
(N)	1·00	1·07
(O)	1·27	1·32
(F)	0·43	0·40

Table 4. *A comparison of results for the copper content of dry butterfat samples by the wet-ashing method and by the simplified method*

Sample	Copper p.p.m.	
	Wet-ashing method	Simplified method
(1)	0·05	0·06
(2)	0·08	0·09
(3)	0·19	0·24
(4)	0·60	0·77
(5)	0·05	0·03
(6)	0·02	0·02

Summary

1. A method is described based on wet-ashing in micro-Kjeldahl flasks for the accurate estimation of iron and copper in 10 g. samples of butter or dry butterfat or in 5 g. samples of cream.

2. An acid extraction method for the routine estimation of copper and iron in dry butterfat is described. This method is shown to give a reliable estimate of the quantities of the metals present.

3. A simplified method for the estimation of iron in cream and butter is described in which the iron is first freed from its protein complex by the addition of thioglycollic acid. Copper is estimated by a similar method, but without the addition of a reducing agent. Both methods give results agreeing reasonably well with the wet-ashing method and are suitable for the routine estimation of these metals in cream and butter.

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352. VARIATIONS IN THE CAROTENE AND VITAMIN A CONTENTS OF CERTAIN NEW ZEALAND BUTTERFATS

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(With 4 Figures)

No systematic work on the vitamin A content of New Zealand dairy products has been published, but occasional observations from various sources indicate that the potency is relatively high (Herrick (1); Crawford, Perry & Zilva (2); Morgan & Pritchard (3)).

Considerable work carried out by the writer whilst a member of the staff of the Dairy Research Institute (N.Z.) in 1935–6 on the carotene (provitamin) and vitamin A values of New Zealand butterfat has remained unpublished, and owing to the renewed interest taken in the nutritional properties of export foodstuffs, these results are now belatedly presented. Although the observations were made nearly 10 years ago, there is no reason to believe that the results would differ materially from those obtaining at the present time.

Much work has been done on the vitamin A content of dairy produce, originally in order to estimate the relative potencies of various food products, but many of the more recent investigations have aimed at tracing the causes of the pronounced seasonal variations which occur. Morgan & Pritchard (3) recorded values for mixed European butters estimated at monthly intervals during one year (Fig. 1). The values were at a minimum in late winter, then showed a sharp rise in spring (May) when the animals were out to grazing, attaining their maximum in early summer (June), thereafter declining slowly until the marked fall in February.

Booth, Kon, Dann & Moore (4), working in England with milk from Shorthorns, found that summer-milk fat had approximately three times the total vitamin A potency of winter-milk fat.

Kon (5) has studied the seasonal variations in total vitamin A activity of bulk milk from sixteen districts in Great Britain, ranging from Aberdeen in the north to Cornwall in the south. He concluded that 'the general seasonal trend is plainly visible for each locality. The graph shows further that even at the same time of the year differences in feeding and management of the cows may produce profound differences in the vitamin A activity of the milk.... In general, summer milk in this country has a potency of 30 or 40 i.u./g. butterfat. In early spring the value may fall to 10 or 20 units.'

Baumann & Steenbock (6), in the United States, found that both carotene and total vitamin A showed a regular decrease at the end of winter, followed by a sharp rise in early summer.

These well-defined seasonal trends in vitamin A content of butterfat were regarded by earlier workers as part of the normal lactation cycle, but it is now known that feeding is by far the most important factor influencing these variations. For example, Gillam, Heilbron, Morton, Bishop & Drummond (7) found, with stall-fed cows, that the carotene

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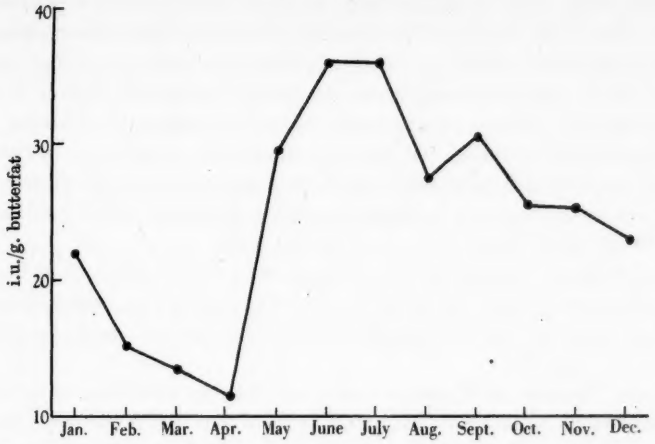

Fig. 1. Average vitamin A potency of mixed European butterfats (after Morgan and Pritchard)

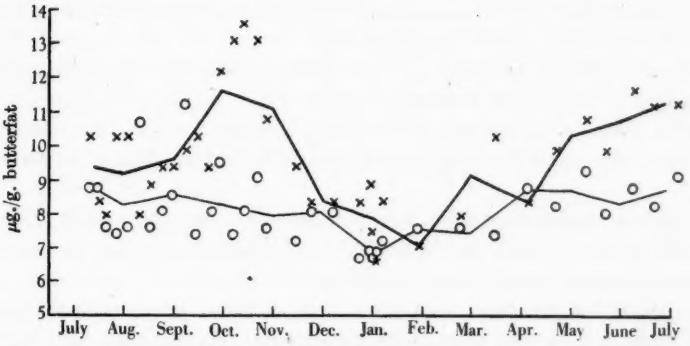

Fig. 2. Carotene and vitamin A in Manawatu butterfats (× = carotene, ○ = vitamin A).

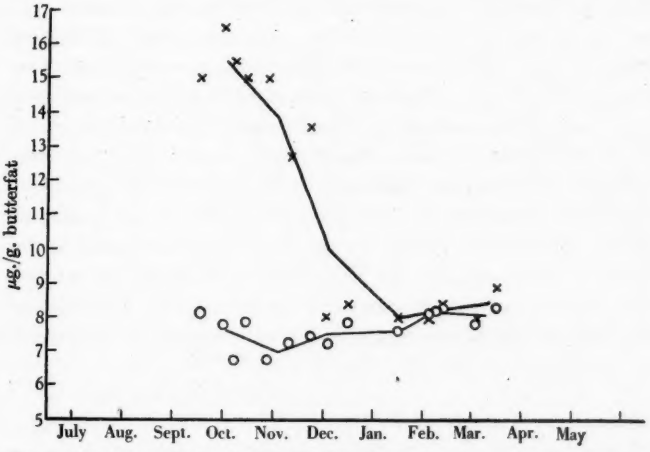

Fig. 3. Carotene and vitamin A in Waikato butterfats (× = carotene, ○ = vitamin A).

content of butterfat was raised by feeding large amounts of carotene-rich products, such as A.I.V. silage or dried grass, but with Shorthorns and Ayrshires the yellow colours of the fats were raised to different levels. In spite of these differences in colour, the total vitamin A values of the milk fats were similar. Baumann, Steenbock, Beeson & Rupel (8) noted that butterfat from Guernseys was much richer in carotene (both during summer and winter) than Friesian butterfat, but lower in vitamin A. In addition to these breed differences, they observed that individual variations within the same breed were 'of the order of 100%'. It is of interest to mention in passing that these workers estimate that only 1–3% of the carotene ingested is secreted in the milk.

Wilbur, Hilton & Hauge (9) found with cows kept under similar feeding conditions that Guernsey butterfat showed twice the carotene content of Ayrshire butterfat, although the total vitamin A potency was similar regardless of the breed of the cow and colour of the butter.

Gillam, Heilbron, Ferguson & Watson (10) observed that the variations in carotene and vitamin A contents of the milk fats of individual cows of four different breeds were large, but these values were more dependent on the diet than on stage of lactation. Guernsey butterfats had both total vitamin A and carotene contents higher than Friesian, Ayrshire and Shorthorn butterfats, but the variations in total vitamin A activity of butterfat from the latter three breeds were scarcely significant.

Crawford *et al.* (2) concluded that 'the breed of the dairy herd seems to have no significant influence upon the vitamin content of the butters'.

Summarizing these typical results quoted from overseas workers, it appears that the principal factors responsible for variations in the vitamin A content of butterfat are the following:

(1) *Individuality.* There are wide differences between cows of one breed. This is not of much practical importance in the New Zealand butter industry, as factory butter is made from mixed cream coming from many hundreds of cows.

(2) *Breed.* Butterfat of cows of the Channel Islands breeds are notable for their higher carotene contents, but it is not clear whether their total vitamin A activity is also higher than for other breeds.

(3) *Seasonal changes.* These are the most striking of the variations noted. The evidence indicates that they are caused by variations in the carotene content of the food, and are therefore of *nutritional* origin. The possibility still exists, however, that there is also a *physiological* (or stage of lactation) effect independent of that due to feeding conditions. This is certainly the case at the beginning of lactation, as Dann (11), Henry, Houston & Kon (12), Stewart & McCallum (13), and others, have shown that immediately after parturition the carotene and vitamin A contents of the colostral fat increase enormously (sometimes to nearly 100 times normal), but decrease rapidly to the usual values within 5–7 days after calving. In addition to this remarkable change in vitamin A concentration there may possibly be other regular but less striking variations associated with the lactation cycle. If such variations are present, they have so far been obscured by the more pronounced seasonal variations resulting from differences in winter and summer food supplies.

EXPERIMENTAL

Four main aspects of the problem were studied, and as the complicating effects due to stall-feeding and the use of concentrates were absent, the results, though of a preliminary nature, explore a somewhat novel field. The particular aspects studied were the following:

(1) *Seasonal variations* in carotene and total vitamin A contents of factory butters from two representative North Island dairying districts (Waikato and Manawatu).

(2) *Effect of breed*—comparison between groups of Friesians and Jerseys under similar lactation, climatic and grazing conditions.

(3) *Colostrum*—examinations of successive samples of colostrum from a Jersey and a Friesian, as well as 'first milkings' from a number of other cows.

(4) *Stage of lactation*—effect on vitamin content.

METHODS

Extraction of butterfat. For most samples the following procedure was adopted: The milk was immediately separated and the cream cooled overnight, then ground with Na_2SO_4 till friable, and again cooled. The fat was then extracted with freshly distilled peroxide-free ether, centrifuged, freed from ether at low temperature, and dried under vacuum. Butter samples (from Waikato) were forwarded as soon as possible after churning, and the fat from a plug in the middle of the block was extracted by a method similar to that described.

The carotene and vitamin A estimations were made without delay, using a B.D.H. Universal Lovibond Tintometer.

Carotene. The non-saponifiable fraction of butterfat was diluted with chloroform to give a reading of 4–6 Y., the results being calculated to Moore's Y.U./g. The proportion of carotene to total carotenoid pigments found (about 85% on the average) was somewhat lower than that recorded by other workers (Gillam *et al.* (7); Dann (11)).

Vitamin A. A chloroform solution of the non-saponifiable residue of an appropriate dilution to give 3–6 B.U. with $SbCl_3$ reagent was used (Davis (14); Dann (11)). Usually three readings were made, the maximum blue development being recorded. The values were calculated to Moore's units (B.U./g.).

All the estimations were made by one worker, though colour matchings in the tintometer were frequently checked by others. It is considered that the values found were reproducible to $\pm 5\%$. Every care was taken to prevent losses of carotene or vitamin A from the sample—e.g. chemical work was carried out in a darkened room, CO_2 was used to replace air wherever practicable, and the determinations were usually made within a day or two of milking.

CALCULATIONS

Conversion of Moore's units to micrograms ($1\ \mu g. = 10^{-6}$ g.)

(*a*) *Carotene.* Moore's Y.U., estimated on the non-saponifiable fraction, were converted to micrograms by using a factor obtained by calibrating the tintometer against dilute solutions of carotene in chloroform.

The carotene, in petroleum-ether solution, was further standardized against 0·1% $K_2Cr_2O_7$ (Ferguson (15)), as its characteristics in chloroform and petroleum ether were different.

Based on colour standards established for B-carotene ($E\,1\%/1$ cm. (463 mμ.)$=2200$) by Ferguson, it was found that in chloroform solution

$$1 \text{ g. carotene} = 2{,}130{,}000 \text{ Y.U.},$$

therefore

$$\text{carotene } \mu\text{g./g.} = \frac{\text{Moore's Y.U./g. (non-sap. fraction)}}{2{\cdot}13}.$$

(*b*) *Vitamin A.* Moore's B.U., estimated on the non-saponifiable fraction, were converted to micrograms by using a factor obtained by calibrating the tintometer against dilute solutions of spectro-photometrically standardized vitamin A concentrates* treated with $SbCl_3$ in the usual way. (The factor differs somewhat from those of certain other workers, apparently because of the relatively small proportion of yellow and neutral tint used in the estimation.)

It was found that

$$\text{Vitamin A } \mu\text{g./g.} = \frac{\text{Moore's B.U./g. (non-sap. fraction)}}{4{\cdot}2}.$$

Conversion of micrograms to international units

In order to compare the total vitamin A potencies of these butters with one another and with results recorded by other workers, the following factors were used:

Carotene: i.u./g. $=\mu$g./g. $\times 1{\cdot}67$ (as defined).

Vitamin A: i.u./g. $=\mu$g./g. $\times 3{\cdot}2$.

It must be pointed out that (1) the factor for carotene (Morton (16)) is based on that obtained with biological tests with rats receiving minimal doses, and that the potency would be probably lower if tested under less extreme conditions. It is also assumed that all of the carotene in butter is in the β-form, whereas Strain (17) has shown that small proportions of the α-isomer are also present, and (2) the factor for converting vitamin A to i.u. though apparently used by the majority of English workers during recent years (Morton (16)); Kon & Thompson (18)) is possibly too low (Jenness & Palmer (19)). If, as suggested by Jenness & Palmer, the factor 4·0 is more accurate, the total vitamin A potencies recorded in this paper will be increased by approximately 15%. On the other hand, the extinction coefficient for carotene recorded by Ferguson (15) on which values recorded herein are based is lower than that recorded by some later workers.

Results

(1) *Seasonal variations in factory butters*

Results for butters from a Manawatu and a Waikato factory are given in Figs. 2 and 3. The trends of the results for the two districts are similar, but in general the Waikato butter was somewhat richer in carotene and total vitamin A activity than the Manawatu butter. The curves show that there was a fall in vitamin content in November which continued to reach a minimum in late summer. The change possibly resulted from the hardening up of the pastures following the spring flush, causing a lowering of the carotene content of the grass.

In the Manawatu, supplementary rations—hay, turnips and maize and other green

* Kindly provided by Mr C. B. Radcliffe, Glaxo Laboratories, Bunnythorpe, New Zealand.

crops—were fed from early in February, but in the Waikato little extra feeding was needed as there was an exceptionally good growth of grass throughout the season.

Comparison of the New Zealand figures with those reproduced from Morgan & Pritchard's paper (Fig. 1) shows that there was only a small decline in the vitamin potency of European butterfats during the hottest period (August), and the values were at their highest throughout summer when the New Zealand values were at their lowest.

On the other hand, European butterfats have very low vitamin A potencies in late winter and early spring owing to the stall-feeding with concentrates necessitated by the cold winters, but under New Zealand grassland-farming conditions, a peak in vitamin A potency is attained during the winter.

(2) *Breed differences*

In Fig. 4 a comparison is recorded of changes in carotene and total vitamin A activity of two groups of cows, one of five Friesians, and one of five Jerseys. All cows were of similar ages and calving dates, and were maintained under typical grassland-farming conditions with a plentiful supply of food.

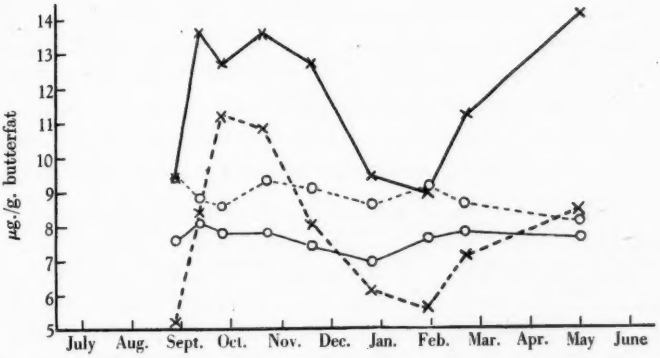

Fig. 4. Carotene and vitamin A in Jersey and Friesian butterfats (Manawatu district). (× = carotene; ○ = vitamin A; continuous line = Jerseys; broken line = Friesians.)

The Jersey butterfats were on the average only about 6% richer in total vitamin A activity than the Friesians, but contained approximately 50% more carotene. These animals were grazed in the Manawatu district, and showed similar seasonal trends to those of the main herd (Fig. 2), which was made up of about 55% Jerseys, 30% Friesians and 15% Ayrshires. The Waikato herds (Fig. 3) would have contained an even higher percentage of Jerseys, which might in part explain the higher carotene and vitamin A values.

(3) *Colostrum*

The remarkable amounts of carotene and vitamin A secreted in the colostral fat is shown in Tables 1 and 2.

Table 1. *Carotene contents of colostral fats from cows at first milking*

	Carotene μg./g. butterfat
Jerseys	168, 183, 118, 157, 249
Friesians	120, 39 (also 59 for a 'second milking')
Ayrshire	56

The values, as found by other workers, are high, being roughly 10–20 times those found in normal milks.

Table 2. *Change in carotene and vitamin A contents of colostrum from a representative Friesian and Jersey cow during first week's milking*

Results expressed in μg./g. butterfat

Days after calving	Sampling	Carotene		Vitamin A	
		Jersey	Friesian	Jersey	Friesian
1	a.m.	—	39	—	86
	p.m.	157	18	54	57
2	a.m.	152	9	44	41
	p.m.	96	8	36	20
3	Morning and evening samples combined	74	6	26	15
4		38	8	18	8
5		26	3	15	7
6		14	3	12	7
7		15	3	10	7
8		14	3	11	8

The high carotene and vitamin A values persisted for only 4 or 5 days after parturition. As producers should not include milk from recently calved cows in their factory supplies until a week after parturition, this factor would be of minor importance in contributing to the high vitamin A potency of spring butterfat.

(4) *Relation of stage of lactation to vitamin A potency of milk fat*

For a study of this phase of the subject, vitamin A activities of butterfat from Jersey cows on the same feed, but of different calving dates, were compared. The comparison was made in February and March on the butterfat from the mixed milk of 'spring-calver' and 'autumn-calver' cows. The former were thus well in the lactation cycle, and the latter at the commencement of lactation. The results given in Table 3 show that there was no appreciable difference in vitamin potencies of the butterfat from the two groups of cows.

Table 3. *Comparison between vitamin potencies of 'spring'—and 'autumn'—calvers (Jerseys)*

		Carotene (μg./g. butterfat)	Vitamin A (μg./g. butterfat)	Total vitamin A (i.u./g. butterfat)
13. xii. 36	'Spring-calvers'	8·9	7·6	39
	'Autumn-calvers'	9·4	7·8	41
6. iii. 36	'Spring-calvers'	11·2	7·8	44
	'Autumn-calvers'	10·3	7·4	41

Since influences due to feed, climate and management were common to both groups, it may be concluded that the seasonal variations reported earlier in the paper were not affected by stage of lactation, but were purely of dietary origin.

DISCUSSION

A summary of the carotene and vitamin A values of commercial butterfats and butters (82% fat basis) is given in Table 4.

Table 4. *Average carotene and total vitamin A potencies of butterfat and butter*

(Results expressed in i.u./g.)

	Average monthly values				Average yearly values	
	Maximum		Minimum			
Source	Carotene	Total vitamin A	Carotene	Total vitamin A	Carotene	Total vitamin A
European:*						
Butterfat	—	36	—	11	—	25
Butter	—	29	—	9	—	21
Manawatu, N.Z.:						
Butterfat	20	47	13	35	16	42
Butter	16	38	11	29	13	34
Waikato,† N.Z.:						
Butterfat	26	50	13	38	19	44
Butter	21	41	11	31	16	36

* After Morgan & Pritchard(3).

† For 6 months only (October–March), but covering maximum and minimum periods.

The New Zealand average yearly values are not inferior to the European highest average (summer) values, while the maximum values for New Zealand butter are considerably higher. However, Kon(5) showed that in Cornwall, with a climate similar to New Zealand, 45 i.u./g. butterfat was attained in May (spring), while for the same month Guernsey milk fat from Shinfield, Reading, contained 48 i.u./g. It is interesting to find that under somewhat similar conditions of grassland farming to that of New Zealand, English butterfats show total vitamin A potencies of the same order as those found in the New Zealand products examined in the present investigation.

SUMMARY

1. Chemical estimations of carotene and vitamin A in New Zealand butters from two of the principal butter-making districts of the North Island during the seasons 1935–6 show that the total vitamin A potency was fairly high.

2. There were seasonal variations apparently due to nutritional rather than physiological causes. The minimum values (33–37 i.u./g. butterfat) for total vitamin A were found in late summer (February) at the time when the pasture normally tends to dry up, while the peak values (42–53 i.u./g. butterfat) occurred in late winter and spring (July-October). The variations in vitamin A potency with season were in the opposite direction to the variations recorded in the literature for Europe and America. The difference is no doubt due to the practice of stall-feeding in these countries in contrast with the all-the-year-round grazing commonly practised on dairy farms in New Zealand. The spring flush of grass is also later in the season in Europe than in New Zealand.

3. The more deeply-coloured Jersey butterfat was only slightly richer in total vitamin A potency than Friesian butterfat.

4. Contents of carotene and vitamin A in the fat of colostrum were very high, but reached normal values within 4 or 5 days after parturition.

The writer is indebted to Mr W. A. Boucher for collecting the Waikato samples, and to Mr G. A. Colpman for help with sampling from the Massey College herd; also to Prof. W. Riddet, Dr F. H. McDowall, Mr A. K. R. McDowell and Dr F. B. Shorland for helpful criticism and advice.

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353. 'MALTY' FLAVOUR IN STARTER AND BUTTER

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One of the most troublesome defects occurring in fresh butter is the so-called malty flavour, which was formerly quite common. It usually first appears in the starter and is transferred to the butter during churning. 'Malty' flavour is caused by micro-organisms. Weigmann (1) in 1899 was the first to show that malty flavour is due to micro-organisms and to isolate an organism which produced a marked malty flavour. He reports: 'Der Geruch ist so intensiv, dass er sich nicht nur ganz und gar auf die Butter überträgt, sondern auch in die Luft des Aufbewahrungsraumes überträgt.' After cultivation on agar for 4 years the organism still produced the malty flavour.

Sadler (2) described an organism, isolated from milk, which produced a caramel flavour before coagulation of the milk. This flavour may be identical with the malty flavour. Sadler's organism resembled *Str. lactis*. In another paper Sadler (3) described a coccus which produced a caramel odour and fermented the same sugars as *Str. lactis*. The source of the taste and odour were not determined. Hammer with his collaborators (4, 5) considers the organism to be identical with a strain of *Str. lactis* which he described, and suggested the name *Str. lactis* var. *maltigenes*. Weigmann (6), however, considers that the malty flavour is formed by degenerated lactic acid bacteria. Sadler (7), in 1928, published an investigation in which he claims that the malty aroma-producing substance is ether-soluble.

In the same year Kelly (8) reported on four diplococci producing a caramel taste. Their behaviour in different sugars was not uniform, and two of them fermented arabinose, raffinose and starch, but not dextrin. Nevertheless, the author regards them as *Str. lactis* var. *maltigenes*, since they coagulate milk rapidly and resemble *Str. lactis* in their morphology and ability to ferment sugars and alcohols. Leitch (9) mentions that the organism producing malty flavour appears in the milk of certain cows.

Heucke (10) describes malty flavour-producing diplococci which never grow in chains. He failed to find them in green plants, but frequently in malts and sometimes in butter and soil. In milk they appeared to be more common in winter than in summer. They coagulated milk even at relatively low temperatures (6–8° C.). Heucke regards them as members of the *Str. lactis* group and states that the ability of bacteria to develop malty flavour is not impaired by continued cultivation in the laboratory. He also claims that the flavour is produced from lactic acid and from all the sugars and alcohols fermentable by *Str. lactis*. Stocker (11) has isolated from milk, starter and butter several species of bacteria producing malty flavour, and he regards them as species of *Str. lactis*. He assumes the malty flavour to arise from casein.

In addition to bacteria of the *Str. lactis* type other micro-organisms have been found to form aroma substances of this kind. Thus Baumann (12) reports the isolation of two species of *Str. faecium* and two species of *Str. bovis* which cause a disagreeable malty smell and taste in milk. One of these species which was transferred daily from milk to milk,

entirely lost its aroma-forming ability after the twentieth transfer. The same organism when grown for a year produced the aroma in undiminished strength. Further, Tracy & Ramsey (13) have observed a pathogenic micrococcus of *Staph. aureus* type which was able to give rise to a malty flavour. When growing with *B. subtilis* it developed a stronger malty flavour than when grown alone, but the acid-forming bacteria retarded its development.

The substances responsible for malty flavour in starter have not been previously described, but in 1944 Virtanen & Nikkilä (14) ascribed it to acetaldehyde. The bacterium causing the formation of acetaldehyde and the malty flavour was shown to be very similar in character and activity to the aroma bacteria, but it produced acetaldehyde in addition to the aroma substances. Normal aroma bacteria do not form acetaldehyde from pyruvic acid although they form aroma substances from it (15). Accordingly, neither sodium bisulphite nor dimedon affect the course of fermentation with these bacteria. The observations of Werkman & Silverman (16) made with an enzyme preparation of *Bact. aerogenes* led to a similar result. The 'malty' coccus, on the other hand, forms acetaldehyde from pyruvic acid, and it seems obvious that the formation of acetaldehyde follows the same course as that of the aroma substances. If diacetyl is formed through the condensation of two $CH_3.CO$ radicals, it can be assumed that acetaldehyde is formed from this radical. Werkman (17) presumes, on the basis of his latest observations with *Aerobacillus polymyxa* concerning the formation of acetaldehyde in addition to aroma substances, that the formation of acetylmethylcarbinol and diacetyl respectively can take two different courses (18). No convincing evidence for this conception has so far been produced.

In 1937 Virtanen & Tarnanen found extensive formation of acetaldehyde in some starters without the addition of substances binding acetaldehyde. In view of the fact that other investigators have not found acetaldehyde, and since in our laboratory this substance was usually not detected, this finding remained obscure. The constant property of the 'malty' cocci to form acetaldehyde now makes the position clear in that these cocci were evidently present in the starters in which Virtanen & Tarnanen found acetaldehyde.

The malty flavour is well known in Finland as a butter defect, and it has sometimes been found to occur in Edam cheese. The defect usually appears in butters from the same plant and its prevention has often been troublesome, since once the organisms producing malty flavour have contaminated the dairy it is difficult to keep them under control. It has been observed in dairies that when a relatively high ripening temperature for the starter is used the malty flavour readily appears even if the initial contamination was slight. Acid formation by the starter is usually retarded and the malty flavour then appears, but the test for aroma substance (Voges-Proskauer, V.-P.) nevertheless gives high values.

Experimental

The cause of malty flavour

From a malty-flavoured starter a Gram-positive *Streptococcus* was isolated on whey-yeast extract-citrate agar. It appears as a diplococcus or forms chains depending on the culture medium and produces a strong malty flavour and taste (14). It is 0·8–1·2μ in size and oblong in form. The colonies formed on whey-yeast extract-citrate agar are round, 0·5–1 mm. in diameter, light grey, rising in the centre, smooth-edged and slightly slimy.

The colonies are often so thin that the light transmitted through them seems coloured. The organism is referred to as the 'malt coccus'.

The temperature curve of 'malt coccus'

Seven flasks each with 300 ml. of sterilized milk were inoculated with 3% of a pure culture of 'malt coccus'. The 500 ml. Erlenmeyer flasks were kept at different temperatures and the coagulation of milk was noted. After 22 hr. the cultures kept at 30 and 37° began to grow thick; the titratable acidity of each culture was determined by the Soxhlet-Henkel method and the *p*H electrometrically with quinhydrone. Simultaneously an aroma test was made (V.-P.). The results are shown in Table 1.

Table 1

No. of exp.	Temp. ° C.	SH°*	*p*H	V.-P.
1	18	14·8	5·75	4
2	26	22·8	5·11	4
3	30	26·4	5·06	4
4	37	27·5	5·00	4
5	40	21·4	5·24	3
6	45	11·0	6·10	3
7	50	10·0	6·17	3

* ml. *N*/4 NaOH/100 ml. milk.

The 'malt coccus' thus sours milk most rapidly at 35–37° C.

The formation velocity of malty flavour in milk cultures

Sterilized milk was inoculated with the milk culture of the 'malt coccus' (3%), and the formation of malty aroma and flavour was determined by taste and smell. The increase in acidity and the development of normal aroma substances were also determined. Table 2 gives the experimental results obtained.

Table 2

	2 hr. after inoculation			4 hr. after inoculation				6 hr. after inoculation			
No. of exp.	SH°	*p*H	Malty flavour	SH°	*p*H	V.-P.	Malty flavour	SH°	*p*H	V.-P.	Malty flavour
1	8·4	6·33	+ +	9·0	6·30	2	+ +	9·6	6·16	2	+ + +
2	8·4	6·34	+ +	9·4	6·26	2	+ +	10·0	6·12	2	+ + +
3	8·0	6·36	+	8·8	6·34	2	+ +	10·0	6·13	2	+ + +

The malty flavour and taste were graded as follows:

\+ = weak; + + = moderate; + + + = strong.

Acetaldehyde as a producer of malty flavour

On steam distillation of malty-flavoured butter or starter prepared with pure culture of the 'malt coccus' its characteristic aroma was found to distil over quite readily. Even before the distillate began to drop from the condenser a pronounced malty flavour could be detected and the highest concentration of malty flavour was found in the first few ml. of distillate. The distillate gave a strong reaction of acetaldehyde (19, 20). Acetaldehyde was found in some milk cultures of the 'malt coccus' to the extent of about 1 mg./100 ml. Pure acetaldehyde added to milk gave a typical malty flavour.

Formation of acids in milk culture of malt coccus

One litre of sterilized skim milk (1·5 l. Erlenmeyer) was inoculated with 3% of the 'malt coccus' culture and the fermentation allowed to proceed for 2 days at 26°, the acidity reaching *p*H 5·02. The lactic acid (21, 22) volatile acids (23) and alcohol (24, 25) in the fermentation solution were determined. The results are presented in Table 3, the quantities being calculated per 100 ml. of fermentation solution.

Table 3

Lactic acid	387·5 mg.	82·7% of the total of acids
Volatile acids:	82·0 mg.	17·3% of the total of acids
Acetic acid	79·3 mg.	97·8% of volatile acids
Formic acid	1·8 mg.	2·2% of volatile acids
Alcohol	35·0 mg.	

The effect of the 'malt coccus' on normal starter at different temperatures

The following series of experiments included milk cultures of normal starter (mark F_{51} of the Laboratory of Valio), pure culture of 'malt coccus' (mark M) and the mixture of both (mark F_{51}+M) in the ratio 1 to 1. The cultures were grown at 16–18, 26 and 30° C. and transferred 6 times, the amount of inoculation being 3%.

The results of the experiments are shown in Table 4.

Table 4

Mark	No. of transfers	Temperature 16–18° C. SH°	*p*H	V.-P.	Malty flavour	Temperature 26° C. SH°	*p*H	V.-P.	Malty flavour	Temperature 30° C. SH°	*p*H	V.-P.	Malty flavour
F_{51}	1	36·0	4·53	3	–	39·4	4·53	3	–	41·0	4·50	3	–
F_{51}+M	1	30·4	4·97	4	+	35·0	4·84	3	+	36·6	4·80	3	+
M	1	16·0	5·85	4	+ + +	16·4	5·80	4	+ +	18·8	5·50	4	+ +
F_{51}	2	27·5	4·80	3	–	37·0	4·50	3	–	41·0	4·48	3	–
F_{51}+M	2	19·5	5·50	3	+	25·1	5·10	3	+ +	33·7	4·75	3	+ +
M	2	13·2	5·95	4	+ +	15·5	5·75	4	+ + +	18·9	5·40	4	+ + +
F_{51}	3	34·8	4·59	2	–	39·6	4·47	2	–	35·0	4·55	2	–
F_{51}+M	3	21·5	5·41	3	+	28·3	4·92	3	+	29·8	4·91	2	+
M	3	11·4	6·10	4	+ +	17·3	5·53	4	+ +	21·8	5·16	4	+ +
F_{51}	4	35·2	4·55	3	–	40·0	4·48	3	–	35·9	4·48	3	–
F_{51}+M	4	22·8	5·30	4	+	30·0	4·92	4	+ +	35·2	4·80	4	+ +
M	4	13·8	6·01	4	+ +	20·3	5·40	4	+ + +	24·7	5·09	4	+ + +
F_{51}	5	39·2	4·50	3	–	43·0	4·45	3	–	42·0	4·50	3	–
F_{51}+M	5	21·8	5·48	4	+	30·4	4·92	4	+	35·9	4·80	4	+ +
M	5	13·0	6·07	4	+ +	17·2	5·68	4	+ +	19·6	5·38	4	+ + +
F_{51}	6	37·6	4·55	3	–	41·0	4·50	3	–	40·0	4·50	3	–
F_{51}+M	6	20·5	4·48	4	+ +	29·8	4·82	4	+ +	30·5	4·92	4	+ +
M	6	12·5	6·10	4	+ + +	16·5	5·65	4	+ + +	19·5	5·45	4	+ + +

The degree of acidity (SH°) of the 'malt coccus' culture was consistently lower than that of normal starter. Also in normal starter the 'malt coccus' caused considerable weakening of the acid-forming power. The malty flavour, even of mixed culture, did not disappear nor diminish during continued growth under these conditions. With increasing temperature the malty aroma became more pronounced. Both the pure and mixed cultures of 'malt coccus' gave rise to a stronger V.-P. reaction than normal starter.

Later we found that the malty flavour weakens and finally disappears from starter kept at low temperatures, if the starter, free from 'malt cocci', was used liberally and the ripening temperature kept low. The results of his investigations with 'malt cocci' will be published later by Nikkilä.

SUMMARY

A Gram-positive, aroma bacterium, the so-called 'malt coccus', has been isolated from malty-flavoured starters. It coagulates milk most readily at 35–37° C. The 'malt coccus' differs from the aroma bacteria mainly in that it forms acetaldehyde from pyruvic acid, evidently through carboxylase. Acetaldehyde is the chief factor in the formation of malty flavour. The 'malt coccus' sours milk feebly. If starter is heavily contaminated with the 'malt coccus', the acidity (SH°) is reduced but the aroma reaction (V.-P.) increases. The malty flavour persists in the starter during transfers and becomes more marked with rising temperature. The 'malt coccus' retains its aroma-forming ability for long periods in the laboratory.

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354. THE APPLICATION OF FAT-ACIDITY DETERMINATION TO A STUDY OF LIPOLYTIC FLAVOUR DEFECTS IN CHEDDAR CHEESE

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During the ripening of Cheddar cheese, the chemical changes which take place in the fat are slight in comparison with the extensive proteolysis. However, the importance of fat hydrolysis in certain other types of cheese is now generally recognized. Besides, increasing attention, which has been given to milk lipase, has emphasized a second aspect of cheese fat degradation. These factors have led to a renewed interest in the chemistry of the fat phase of cheddar cheese.

Reported studies have dealt with two aspects of cheese-fat degradation. Determination of volatile acids as an index of both the kind and the extent of decomposition of Cheddar-cheese fat was used by Lane & Hammer (12, 13), made quantitative by Hiscox *et al.* (4, 5, 6) and recently shortened from 40 to 4 hr. by Smiley *et al.* (15). On the other hand, the simpler but more arbitrary titratable fat acidity has been used by Lane & Hammer (12, 13), by Babel & Hammer (2) and by Irvine (11).

In continuation of our studies on milk lipase in relation to the development of lipolytic flavour defects in raw-milk Cheddar cheese, the application of fat-acidity determination has been extended, and further chemical background information on cheese quality has been obtained.

I. Fat-acidity determination

The first step in the determination of titratable acidity is the isolation of dry filtered fat from the cheese. Lane & Hammer (12) accomplished this by submitting a mixture of cheese and sand to pressure, separating the fat, and finally filtering it through a dry paper. Irvine (11) greatly simplified the procedure by leaving ground cheese to oil off in tall beakers set in warm water. The melted butterfat was used directly.

For several years a procedure somewhat similar to Irvine's has been used in the Science Service laboratories. It consists of placing grated Cheddar-cheese sample in a suitable funnel provided with a folded filter-paper and supported in a 65° C. oven. At the elevated temperature the cheese fat melts, runs down and filters into a small receiving flask. The temperature selected does not appear to be critical and 100° C. has been used successfully, shortening considerably the filtration period.

A 10 g. portion of the dry isolated cheese fat is titrated in boiling 95% neutral ethanol using 8 drops of phenolphthalein indicator. The titration is essentially that which has been deleted from the fifth and sixth editions of *Methods of Analysis* (A.O.A.C. (1)), but is still useful in certain phases of dairy science. The result is expressed as ml. of $N/10$ NaOH per 10 g. fat and is termed the acid degree.

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II. FAT ACIDITIES ON FIRST-GRADE EXPERIMENTAL CHEESE

Fat-acidity determinations, by the method just described, were made approximately at weekly intervals over a period of 1 year. The experimental cheese were made according to accepted Canadian methods of manufacture from raw milk of known quality. Samples for analysis were taken when the cheese were 2–4 weeks old in order to approximate the maturity of commercial cheese when offered for grading.

The results for thirty-eight samples are summarized in Table 1. The average titratable acidity for this series of cheese is 1·0 ml. of $N/10$ NaOH per 10 g. fat. This value may be regarded as a reliable reference against which commercial or experimental cheese can be compared.

Table 1. *Acid degree of first grade experimental cheese*

Acid degree	0·8	0·9	1·0	1·1	1·2	1·3	1·4
No. of samples	5	8	9	4	6	5	1

Irvine (11) provided data on three Canadian first grade commercial Cheddar cheese 4–7 weeks old. Bringing his results to a basis of 10 g. fat, their average is 1·3. The average of six values given by Lane & Hammer (12) for experimental American Cheddar 2 days old is 1·1. These values, although a trifle higher, are within the range given in Table 1.

III. FAT ACIDITY OF PASTEURIZED MILK CHEESE

An acid degree of 1·0 for cheese fat is considerably higher than that for normal sweet-cream butter (12) or pasteurized-milk cream (16). This observation points to the action of milk lipase. In order to assess the contribution of milk lipase in the decomposition of cheese fat, Lane & Hammer determined fat acidities on American Cheddar made from pasteurized milk. The average of six results for 2-day-old cheese is 0·9, somewhat lower than for unpasteurized-milk cheese, and increasing only slightly with maturity in contrast with the latter. An average acid degree of 0·8 for cheese 1 month old is given by Babel & Hammer (2).

Because the manufacture of Canadian Cheddar cheese differs from the American method in that a higher amount of acid is developed during the initial stages and a lower-moisture cheese is produced, four experimental cheese were made from pasteurized milk to ascertain any differences in fat acidity. The average acid degree obtained was 0·6 for cheese 2–4 weeks old. This approaches more nearly the value for sweet-cream butter and at the same time indicates the extent to which milk lipase takes part.

IV. LIPOLYSIS IN EXPERIMENTAL CHEESE FROM AGITATED MILK

Work from our laboratories (7, 8) has already established, on the basis of organoleptic grading, that rancid and unclean lipolytic flavour defects can be induced in Cheddar cheese by submitting warm cheese milk to vigorous agitation. Titratable acidity of cheese fat was now determined in order to follow chemically the extent of hydrolysis resulting from lipase action under these conditions. Determinations were made on the cheese when removed from the press at 3 days and again after 30 days' storage at 60° F.

Essential data are summarized in Table 2. Series A represents normal comparative vats corresponding to series B in which cheese milk was the same but submitted to agitation at 86° F. Each result is the average of two similar vats.

Table 2. *Extent of lipolysis in Cheddar cheese*

No.	Treatment of milk	Acid degree of cheese fat		
		At 3 days	At 30 days	Difference
1A	—	1·0	1·3	0·3
1B	15 min. agitation	15·4	16·6	1·2
2A	—	0·9	1·2	0·3
2B	10 min. agitation	11·2	12·7	1·5
3A	—	1·1	1·1	0·0
3B	5 min. agitation	3·4	3·8	0·4
4A	—	1·1	1·2	0·1
4B	3 min. agitation	2·9	3·1	0·2

It is apparent that a high acid degree was obtained on cheese fat where milk had been submitted to vigorous agitation. The longer the period of agitation the higher was the acid degree obtained. This finding confirms on a chemical basis our previous work on the action of milk lipase. Similar results were obtained by Lane & Hammer (13) who reported higher fat acidities in cheese, made from skim milk plus homogenized cream, at 4–6 months' maturity.

It is also interesting to note that extensive lipolysis took place before the cheese were removed from the press. The increase in acid degree during the next 30 days, although slightly higher in the B than the A series, was slight in comparison. In other words, milk lipase does not show much activity after the cheese is made, probably on account of unfavourable hydrogen-ion concentration (3, 14). This is in agreement with the data obtained from experiments on the keeping quality of Cheddar cheese with unclean and rancid flavours (9).

V. Fat acidity of commercial Cheddar cheese

Our primary interest in the study of cheese-fat acidity was to determine its application to commercial Cheddar cheese. For several reasons, however, it was not possible to obtain a sufficiently large number of samples to undertake a detailed study of Canadian cheese. This is planned for the future. Nevertheless, a preliminary survey has been made.

A series of fifty-five 3–4 oz. samples, selected to include a large proportion of cheese degraded for unclean and rancid flavour, was obtained. The age of the cheese ranged from 16 to 93 days. Upon analysis the cheese samples were arranged in order of increasing fat acidities. These ranged from 1·2 to 4·6 $N/10$ NaOH per 10 g. fat with two exceptionally high values of 24·8 and 26·5. It should be noted here that if the amount of cheese sample was insufficient to obtain 10 g. of fat, a lesser amount was titrated and calculated to a 10 g. basis. The results are summarized in Table 3.

Table 3. *Acid degree of commercial Cheddar cheese*

Acid degree	No. of samples	No. of undergrade
1·2– 1·5	12	2
1·6– 2·0	14	7
2·1– 2·5	10	8
2·6– 3·0	5	3
3·1– 3·5	4	4
3·6– 4·0	5	5
4·1– 4·5	2	2
4·6– 5·0	1	1
5·1–27·0	2	2

The series as a whole contained 62% undergrade cheese. Only two out of twelve cheese with an acid degree between 1·2 and 1·5 were below first grade. The incidence of undergrade cheese increased to 7 out of 14 for cheese with acid degree of 1·6–2·0 and to 8 out

of 10 with acid degree of 2·1–2·5. All the cheese with an acid degree of 2·8 or higher were undergrade.

These figures clearly indicate that the higher the acid value of cheese fat, the greater is the incidence of unclean and rancid flavour defects. Thus milk lipase appears to be implicated in the development of rancid and unclean flavours in commercial Cheddar cheese. It is noteworthy, however, that with the exception of two high values most acid degrees were lower than would be expected from the experimental values in Table 2. It is obvious, of course, that the conditions under which lipolysis takes place in experimental cheese are not entirely the same as those in factory cheese, and complete correspondence of acid degrees is not necessarily expected. In fact, further evidence has been obtained on this topic and is presented in the following section.

VI. Fat acidity and lactic acid development in Cheddar cheese

A study of the relation between the development of lactic acid and lipolytic flavour defects in Cheddar cheese is reported in greater detail elsewhere (10). Each pair of comparative cheese, listed in Table 4, was made from the same milk. To the milk of the N-series a small amount of starter was added the night before and some lactic acid was allowed to develop by holding the milk at 60° F. The M-series received 3% starter in the morning only, but was otherwise identical. Lipase action was induced in both series by homogenizing a definite proportion of milk in each vat in the morning. Acid degrees determined after 30 days and organoleptic flavour scores at 2–3 weeks are summarized in Table 4.

Table 4. *The effect of lactic acid development and acid degree in Cheddar cheese*

Exp. no.	Age of cheese (days)	Flavour score	Acid degree
1N	14	39·5	2·8
1M	14	37·0	2·3
2N	21	39·5	3·3
2M	21	39·0	3·3
3N	22	40·0	5·6
3M	22	38·5	6·9
4N	15	39·0	6·1
4M	15	39·5	7·5
5N	14	38·5	9·3
5M	14	37·0	11·1
6N	22	38·0	11·1
6M	22	37·0	10·4
7N	22	40·0	8·5
7M	22	38·0	9·5
Average N-series		39·2	6·7
Average M-series		38·0	7·3

With the exception of vat 4N, cheese made from milk to which starter was added the night before scored higher on flavour than the corresponding cheese of the M-series. In view of the known inhibition of the action of milk lipase in an acid medium, a hasty conclusion might be drawn that the improvement in flavour score was due to this effect. Fat acidity, however, indicated a different picture. Although acid degrees of the N-series are slightly lower than those of the corresponding numbers of the M-series, this appears to be a minor feature. It is more interesting to note a high fat acidity coincident with a first-grade flavour score, as, for example, in cheese 4N and 7N. This observation points to the importance of the conditions under which lipase action takes place. Its significance, however, remains to be assessed on the basis of further studies.

Discussion

The titratable acidity of cheese fat measures primarily the water-insoluble fatty acids. If one assumes a uniform behaviour of milk lipase towards its substrate, such a measure is indicative of total lipolysis. A consistent picture of the action of milk lipase in cheese, as reflected by the experimental data presented, supports that assumption. A note of inconsistency, however, has been included in the last section to serve as a reminder that caution is necessary in interpreting data. Used judiciously, fat acidity can be of value in the study of the chemistry of cheese-fat degradation.

Summary

1. A simple method of determining the acid degree of cheese fat has been described.

2. An average value of fat acidity on a series of thirty-eight samples of experimental Canadian first-grade Cheddar cheese, covering a period of about a year, has been established at 1·0 ml. *N*/10 NaOH per 10 g. for cheese 2–4 weeks old.

3. An average acid degree for four experimental cheese from pasteurized milk was found to be 0·6.

4. A high acid degree was obtained on cheese fat made from milk in which lipase action was induced by vigorous agitation; the longer the period of agitation the higher was the acid degree. Extensive lipolysis took place at some stage before the cheese was removed from the press. Subsequent increase in acid degree was slight.

5. Fat acidities on a series of commercial Cheddar cheese, selected so as to include a high proportion of cheese degraded for unclean and rancid flavour, ranged from 1·2 to 4·6 with two exceptionally high values of 24·8 and 26·5. The incidence of undergrade cheese increased as the acid degree increased. All cheese with an acid degree of 2·8 or higher were below first grade on flavour.

6. Cheese made from milk, in which acid was developed by the addition of a small amount of starter the night before and which was partly homogenized, showed a high acid degree although the flavour was quite good—often first grade. Similar cheese, but with 3% starter added in the morning, showed only slightly higher fat acidity and scored lower for flavour. These findings cannot be explained as a simple inhibition of the action of milk lipase by acid development.

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355. CHEESE STARTER 'RECOVERY' AFTER ATTACK BY BACTERIOPHAGE. (STRAIN DOMINANCE IN MULTIPLE-STRAIN STARTERS)

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Introduction

It sometimes happens that a commercial cheese starter which 'fails' in the vat may, on continued daily subculture, behave normally after a few days. Cheese-makers call this starter 'recovery', but a search of the literature yielded no relevant information. It is probable that a commercial starter originally consists of a mixture of several strains of lactic streptococci. Many of these strains are fully active and capable of producing the acidity necessary for cheese-making, and it is reasonable to suppose that owing, for example, to different growth rates or responses to temperature of incubation, one of the strains becomes predominant in the starter on continued culture.

If such a predominant strain in a commercial starter fails to produce normal acidity after the selective destruction of that strain by phage, one or more of the other strains, unaffected by the phage, may replace the strain which has 'failed'. Although these subordinate strains may originally have been responsible only for a small part of the total acidity produced, they may after subculture for a few days become mainly responsible for the 'activity' of the starter. The starter then appears to have 'recovered'.

The strains remaining after the predominant one has phaged may be 'slow' and unsuitable for cheese-making, and, indeed, 'slow' strains are normally present in all 'active' starters subcultured daily. The 'resistant' secondary strains which sometimes develop after a single strain has phaged may produce the acidity necessary for cheese-making and bring about the true 'recovery' of a starter, but 'resistant' strains are usually sluggish. There appears at present to be no method of forecasting the behaviour of the residual strains after the predominant one is eradicated.

It was thought that, by mixing unrelated phaging strains of lactic streptococci suitable for starter and using the corresponding phage to eliminate each strain successively from the 'mixed' starter, it might be possible to ascertain the behaviour of strains in a multiple-strain starter and to elucidate starter 'recovery'.

I. Strains for experimental starters

Three fast acid-producing strains, 1P5, 144F and 318B/27, were chosen, which were attacked by their specific phages, and not by those of the other two strains (1). Two other strains, L314/1 and 170/1, isolated from different sources but attacked by the phages of 1P5 and 318B/27 respectively, were selected to replace the latter organisms when desired, since their rates of growth and other characteristics would probably be different and their use would facilitate the investigation.

The phage-organism relationships of the strains used are given in Table 1 A and B. Tests for cross-phaging were made in milk by an 'activity' test (2) or phage detection

test (3). Approximately $\frac{1}{10}$ ml. of a purified phage suspension (filtered through a 'Seitz' filter), prepared in yeast-dextrose broth (not in whey), was added to 25 ml. of the inoculated strain under test. An equal volume of sterile yeast-dextrose broth was added to the control sample to compensate for the stimulatory effect of the broth medium. The acidity was determined when the control milk had coagulated, usually after incubation at 30° C. for $5\frac{1}{2}$–8 hr. Under the conditions of the test, acid production was arrested when phaging occurred.

Table 1

A. *Phage-organism reactions of strains selected as components of experimental starters*

		Phages added				
Strains	Control	1*p*5	144*f*	318*b*/27	*l*314/1	170/1
1P5	5·2	**2·1**	4·9	4·9	**2·2**	5·0
144F	5·1	5·1	**2·2**	5·0	5·0	5·0
318B/27	5·1	5·1	4·9	**2·2**	5·1	**2·6**
L314/1	4·3	**2·1**	4·5	4·5	**2·0**	4·7
170/1	5·3	5·2	5·1	**2·1**	5·4	**2·1**

B. *Summary of cross-phage reaction*

	Phages added				
Strains	1*p*5	144*f*	318*b*/27	*l*314/1	170/1
1P5	+	–	–	+	–
144F	–	+	–	–	–
318B/27	–	–	+	–	+
L314/1	+	–	–	+	–
170/1	–	–	+	–	+

+ = phaging; – = non-phaging.

Key for Tables 1A, 3, 4, 5, 6 *and* 7

(1) The results given are titration figures of the milk 'activity' tests, i.e. no. of ml. of *N*/10 NaOH required to neutralize the acidity of 10 ml. of inoculated and incubated milk—with and without (control) the addition of phage.

(2) The titration figure for the inoculated milk before incubation was approx. 1·9 ml. *N*/10 NaOH.

(3) Figures in heavy type denote phaging considered to have occurred, since acid production, after the addition of phage or whey, has failed to develop. Figures in italic type slight phaging or effect doubtful.

Nascent phage (3, 4) could not be demonstrated, and if irregularities occurred when these strains were mixed they were not due to nascent phage.

II. Preparation and preliminary examination of the experimental starter mixtures

To ensure that the strains in each experimental starter were unrelated, they were mixed in three different combinations only, viz.

Mixture A:	Mixture B:	Mixture C:
1P5	L314/1	1P5
144F	144F	144F
318B/27	318B/27	170/1

Throughout the experiments the starter mixtures and the strains from which they were prepared, were subcultured daily in separated milk using 1% inoculum, and incubated at 30° C. Daily subcultures of the starter mixtures were also incubated at 22° C.

Each starter mixture was prepared as follows: Equal volumes of 24 hr. cultures of the constituent strains were thoroughly mixed and, when first prepared, 1, 0·25 and 0·1%

of this experimental mixture used as inocula in sterile separated milk for 'activity' tests at 30° C. These detection tests were carried out on the inoculated milk (control) and on inoculated milk after the addition of each of the phages attacking the strains used in the mixture. Indications of the two most suitable strengths of inoculum necessary to secure coagulation of the controls in 6–9 hr. and to give suitable conditions for the three different phages to act on each mixture were obtained from these preliminary tests. The results presented in Tables 3–5 are the average of those obtained from the two tests using different strengths of inocula.

The individual strains were plated on yeast-dextrose agar at the time of mixing and the plates counted (Table 2) after incubation at 30° C. for 48 hr. The number of colonies in each of the three strains constituting the experimental starters was therefore known. Although equal volumes of culture from each strain had been used, the numbers of organisms added were not necessarily similar.

Table 2. *Plate count per ml. of milk culture of strains used for experimental starters at the time of mixing*

	Starter mixture		
Strains	A	B	C
1P5	$6{,}100 \times 10^6$	—	$115{,}200 \times 10^6$
L314/1	—	$1{,}760 \times 10^6$	—
144F	$2{,}220 \times 10^6$	$2{,}210 \times 10^6$	$59{,}600 \times 10^6$
318B/27	$4{,}900 \times 10^6$	$33{,}400 \times 10^6$	—
170/1	—	—	$14{,}800 \times 10^6$

It was possible that a direct relationship might exist between the number of organisms of each constituent strain originally mixed to form the experimental starter and the ability of one strain eventually to predominate over the other two after continued subculture. With the exception of mixture C, wide variations in the number of organisms in the inocula did not occur, and it will be shown later (p. 109) that wide numerical differences in the inocula did not ultimately determine the predominance of a strain.

III. Behaviour of strains in a starter mixture on continued subculture

The results of the first 'activity' tests (Tables 3A–5A) on the experimental starters, made immediately after mixing, indicated that each of the three component strains contributed to the acidity produced, since on the addition of each phage the acidity was lower than that of the control starter alone. If the 'activity' test indicated that the acid production of the starter was retarded on adding phage corresponding to one of the component strains but was not significantly affected by the phages for the other two strains, then the strain, corresponding to the phage which inhibited acid production, was probably responsible for most of the acidity produced by the starter. Such a strain was considered to be the predominant one.

The starter mixtures were retested in this way weekly until one of the strains appeared to be consistently predominant and then tested at intervals to detect whether any change had occurred. Since later experimental treatment of the starters depended on a strain becoming predominant, the results are best discussed at this stage.

(*a*) *Predominance of one strain in the experimental starter mixtures after daily subculture*

'Activity' tests showed that in each starter mixture one strain became predominant on prolonged daily transfer (Tables 3B–5B). In mixtures A and C, strain 1P5 was responsible for most of the acid developed during the 'activity' test after subculturing for 2 weeks and 1 week respectively. Even after culture of mixture A for 111 days and of mixture C for 33 days 1P5 remained dominant in these starters. In mixture B strain 144F predominated after 2–3 weeks' propagation at 30° C. When the same mixture was incubated at 22° C., strain 318B/27 also contributed to the acidity produced, and after 7 weeks' propagation strain 144F had not entirely suppressed the other two strains.

(*b*) *Elimination of predominant strains from starter mixtures*

First predominant strain. When it became apparent on continued propagation of a starter mixture that one strain had outgrown the others, the predominant strain was eliminated by phage.

As each starter was propagated at 22 and 30° C. an 'activity' test was set up for both subcultures. The milk inoculated with two different concentrations of starter mixture, as previously described, was divided into two portions, to one of which the phage reacting with the predominant strain was added. A comparison of the results from the two portions showed whether phaging of the predominant strain had taken place. When phaging had occurred, the two lysed cultures which differed only in the strength of the original inocula, were bulked and then reincubated at the same temperature as the original mixtures. These starters, resulting from the lysed mixtures A, B or C, were subcultured daily and called A1, B1 and C1 (the '1' mixtures).

Each '1' mixture was examined 24 hr. after preparation and thereafter at longer intervals over a period of several weeks by an 'activity' test, using the phages corresponding to the three strains originally present in each mixture. From these tests major changes in the proportion of the strains responsible for acidity development could be detected.

With the exception of C1 incubated at 22° C. the '1' mixtures were coagulated 24 hr. after the addition of the eliminating phage.

Second predominant strain. In the first two mixtures, A1 and B1, another strain became predominant and was eliminated by the corresponding phage. These new mixtures were called A2 and B2. From mixture B1, at 22 and 30° C., different strains were eliminated. A mixture C2 could not be prepared since, on continued subculture of mixture C1, the predominance of any one of the original strains was not detected.

The detailed results of the tests on the '1' mixtures (Tables 3D–5D) show that strain 144F contributed mainly to the acidity of mixture A1 at both temperatures, although at 22° C. strain 318B/27 was also partially responsible, and in this respect compares with the results of starter B (Table 4B) in which these same two strains contributed to the total acidity developed after prolonged subculture.

In starter B1 (Table 4D) strain 318B/27 became predominant at 22° C., whilst at 30° C. L314/1 was eventually predominant. The temperature of incubation determined the strain which finally predominated. Since the acid production of strain 318B/27 alone has been shown to be retarded by the corresponding phage at 30° C. as well as at lower temperatures, there would be little chance of this strain remaining undetected had it become predominant in mixture B1 at that temperature.

Tables 3–5. *Predominant strains which develop in experimental starters as indicated by phage reactions of*

Tables 3–5. *Predominant strains which develop in experimental starters as indicated by phage reactions of constituent strains*

Table 3. *Mixture A*

Temp. of starter propagation ...			(1) 22° C.					(2) 30° C.				
Days after strains				Phages added			Incubation period		Phages added			Incubation period
Mixed	Eliminated	Inoculum %*	Control	1*p*5	144*f*	318*b*/27	(hr.)	Control	1*p*5	144*f*	318*b*/27	(hr.)
(A) Preliminary tests on A at the time of mixing (25. vii. 44)												
—	—	1	—	—	—	—	—	5·2	4·5	4·4	4·7	6
—	—	0·25	—	—	—	—	—	5·0	4·2	3·7	4·7	7
—	—	0·1	—	—	—	—	—	4·8	4·1	4·4	4·4	7½
(B) Tests on A after prolonged propagation												
6	—	0·25+0·1	7·3	4·8	7·3	7·2	7½	6·2	3·6	6·2	6·2	7½
14	—	0·25+0·1	5·5	2·6	5·4	5·5	7	5·1	2·2	*4·9*	5·1	7½
20	—	0·25+0·1	5·2	2·3	5·2	5·2	6½	5·0	2·2	*4·8*	5·2	7½
98	—	0·25+0·1	4·7	2·1	*4·3*	4·8	6½	5·0	2·0	4·5	5·0	7
111	—	0·25+0·1	4·8	2·0	4·9	4·9	6½	5·2	2·3	5·1	5·2	6½
(C) Tests on A after addition of phage of first predominant strain (1 P 5)												
28	—	0·25	5·3	2·2	—	—	6½	5·2	2·1	—	—	7
—	—	0·1	5·3	2·2	—	—	7	5·0	2·2	—	—	8
(D) Tests on A 1 after prolonged propagation												
30	2	1	4·9	4·8	3·9	*4·7*	8	4·8	4·7	2·0	4·9	8½
34	6	1	5·7	5·8	4·2	5·3	5½	4·9	5·1	2·1	5·1	6
43	15	0·25+0·1	5·3	5·5	3·6	4·8	6½	5·4	5·4	2·0	5·5	7
56	28	0·25+0·1	5·4	5·5	3·9	4·3	7	5·1	5·1	1·9	5·1	7
77	49	0·25+0·1	5·4	5·6	4·0	4·6	7	5·3	5·4	2·5	5·4	7
(E) Tests on A 1 after addition of phage of second predominant strain (144 F)												
55	—	1	4·7	—	3·7	—	5	4·7	—	2·4	—	6
—	—	0·25	4·8	—	3·2	—	6	4·9	—	2·0	—	6
(F) Tests on A 2 after prolonged propagation												
56	1	1+0·25	4·1	4·4	4·4	2·1	8½	5·1	5·1	5·1	5·1	7
64	8	1+0·25	4·9	4·9	4·9	3·1	6	5·1	3·7	*4·9*	5·0	6½
65	9	1+0·25	4·9	4·9	4·9	3·3	6	4·9	3·8	4·9	4·8	7
74	18	1+0·25	4·6	4·6	4·7	3·0	7	4·7	3·3	4·9	5·0	7
91	35	1+0·25	5·3	5·3	5·3	3·4	6	5·2	3·4	5·4	5·2	6½
99	43	1+0·25	4·8	5·0	4·7	3·5	6½	4·8	2·7	*4·5*	4·8	6½
107	51	1+0·25	4·9	4·8	*4·6*	2·5	6½	5·0	3·2	4·9	5·0	6½
114	58	1+0·25	4·8	4·8	4·9	2·7	6½	5·2	3·3	5·1	5·4	6½

* 0·25+0·1, figures given are average of those obtained for two tests using 0·25 and 0·1 % of inocula respectively.

Table 4. *Mixture B*

Temp. of starter propagation ...			(1) 22° C.					(2) 30° C.				
Days after strains				Phages added			Incubation period		Phages added			Incubation period
Mixed	Eliminated	Inoculum %*	Control	*i*314/1	144*f*	318*b*/27	(hr.)	Control	*i*314/1	144*f*	318*b*/27	(hr.)
			(A) Preliminary tests on B at the time of mixing (8. ix. 44)									
—	—	1	—	—	—	—	—	5·5	**4·9**	**4·3**	**4·7**	5
—	—	0·25	—	—	—	—	—	5·8	5·6	**5·0**	*5·4*	5
—	—	0·1	—	—	—	—	—	5·3	*5·0*	**4·6**	*5·1*	7½
			(B) Tests on B after prolonged propagation									
7	—	0·25+0·1	5·2	5·1	**3·6**	*4·9*	7	5·2	5·2	**3·3**	5·2	7
14	—	0·25+0·1	4·8	4·9	**3·5**	**3·8**	6½	4·8	5·0	**2·7**	5·3	7
19	—	0·25+0·1	5·0	5·1	**4·0**	**4·0**	6½	4·9	5·0	**2·9**	5·0	7
47	—	0·25+0·1	5·7	5·9	**4·1**	**4·9**	6½	5·5	5·3	**2·6**	5·4	6½
			(C) Test on B after addition of the phage of first predominant strain (144F)									
26	—	1	5·1	—	4·0	—	5	4·8	—	**2·5**	—	5
			(D) Tests on B1 after prolonged propagation									
27	1	0·25+0·1	4·1	4·5	4·4	**2·7**	9	4·8	5·0	4·9	**4·1**	9
33	7	1·0+0·25	5·6	5·6	5·6	**3·0**	6½	5·3	5·2	*5·1*	5·5	8
41	15	1·0+0·25	5·7	5·7	5·9	**3·6**	6½	5·5	**4·0**	**5·1**	**5·1**	8
48	22	1·0+0·25	5·3	5·8	5·4	**3·2**	7	4·5	**3·7**	*4·2*	*4·3*	8½
54	28	1·0+0·25	5·6	5·9	5·6	**3·2**	6	5·0	**4·0**	6·4	5·8	7½
68	42	1·0+0·25	—	—	—	—	—	4·8	**3·2**	4·8	4·9	6½
			(E) Tests on B1 after addition of the phage of the second predominant strain (318B/27 at 22° C. and L314/1 at 30° C.)									
56	—	1	5·5	—	—	**3·4**	6	4·6	**3·7**	—	—	7
—	—	0·25	4·4	—	—	4·3	6	3·7	**3·1**	—	—	7
			(F) Tests on B2 after prolonged propagation									
57	1	1·0+0·25	5·3	*5·1*	5·3	5·3	5½	4·7	4·6	4·8	4·6	7½
62	6	1·0+0·25	4·8	**3·9**	5·4	5·1	6	4·7	4·7	4·7	4·9	7½
67	12	1·0+0·25	4·8	**3·2**	5·0	4·8	5½	4·9	4·8	*4·7*	4·8	7
74	19	1·0+0·25	4·5	**2·8**	4·5	4·7	6	4·4	4·3	4·5	4·5	7

* 0·25+0·1, figures given are average for those obtained for two tests using 0·25 and 0·1% of inocula respectively.

Table 5. *Mixture C*

Temp. of starter propagation ...			(1) 22° C.					(2) 30° C.				
Days after strains				Phages added			Incubation period		Phages added			Incubation period
Mixed	Eliminated	Inoculum %*	Control	1*p*5	144*f*	170/1	(hr.)	Control	1*p*5	144*f*	170/1	(hr.)
(A) Preliminary tests on C at the time of mixing (8. ix. 44)												
—	—	1	—	—	—	—	—	5·3	*5·1*	**4·3**	5·3	7½
—	—	0·25	—	—	—	—	—	4·8	**4·1**	**3·8**	*4·6*	8½
—	—	0·1	—	—	—	—	—	3·6	3·6	**3·1**	3·5	8½
(B) Tests on C after prolonged propagation												
7	—	1+0·25	4·7	**2·1**	4·6	4·6	5½	4·8	**2·4**	5·3	5·2	6½
13	—	1+0·25	5·3	**2·6**	5·7	5·5	5½	5·3	**2·7**	5·2	5·5	6
20	—	1+0·25	6·0	**2·6**	5·9	5·8	6	5·9	**2·7**	6·1	6·2	6
26	—	1+0·25	5·7	**2·4**	5·6	5·7	5½	6·1	**2·6**	*5·9*	6·1	6
33	—	1+0·25	7·5	**2·5**	7·2	7·3	5½	6·1	**2·4**	6·0	*5·9*	6
(C) Tests on C after addition of phage of first predominant strain (1 P 5)												
27	—	1	6·8	**2·7**	—	—	6	5·6	**2·7**	—	—	6
—	—	0·25	6·6	**2·6**	—	—	7	5·6	**2·3**	—	—	7
(D) Tests on C1 after prolonged propagation												
28	1	1	—	—	—	—	—	5·1	5·5	5·1	5·4	7½
29	2	1+0·25	5·6	5·6	5·6	5·8	6	4·9	4·9	4·9	5·0	7
30	3	1+0·25	6·1	6·0	*5·9*	6·0	6½	5·4	5·4	5·3	5·8	7
42	15	1+0·25	4·9	5·0	4·8	4·9	6½	4·7	5·0	4·6	5·3	7½
49	22	1+0·25	4·9	4·9	4·9	4·9	6½	4·6	4·8	4·7	4·9	7½

* 0·25+0·1, figures given are average of those obtained for two tests using 0·25 and 0·1 % of inocula respectively.

After strain 1P5 had been eliminated neither of the residual strains (144F and 170/1) in the new mixture C1 (Table 5D) predominated. This result is difficult to explain. Whereas 144F became the predominant strain in mixture A1, after strain 1P5 had been eliminated by phage, strain 144F did not become predominant in mixture C1 after 1P5 had again been removed by phage. Perhaps secondary strains resistant to phage 1*p*5 had arisen which were also able to produce high acidity in milk and overgrow the residual strains (144F and 170/1) originally in the mixture C1. It is also possible that during prolonged cultivation of mixture C1 natural variants of strain 144F or 170/1 may have arisen which, although resembling the original strains in acid production, might differ in other characteristics, e.g. susceptibility to lysis by the phage of the strains from which they arose. Such variants might gain predominance in the mixture, but it would be impossible to trace them by using the phages of the strains originally present in C1. In A2, even after propagation for 58 days at 30° C., strain 318B/27 was not detected. This strain is known to grow less well in competition with others at 30° C. and consequently it may have disappeared entirely. A strain susceptible to lysis by phage 1*p*5 again became predominant in mixture A2, but addition of this phage did not entirely eliminate the acid production of the starter as it had done in mixture A. After phage 1*p*5 had been added to mixture A it is difficult to understand how any organisms normally lysed by this phage could have survived. No trace of strain 1P5 could be detected in mixture A1 when it was tested over a period of 49 days in the presence of phage 1*p*5, yet some variant susceptible to lysis by phage 1*p*5 must have arisen from one of the strains in the mixture after starter A2 had been subcultured for 8 days.

When 318B/27 had been excluded from mixture B1 by phaging at 22° C., L314/1, the only original strain remaining in the mixture, became predominant at 22° C. (Table 4F), but there was no indication that any one of the original strains of the mixture was responsible for the acid production in B2 at 30° C. 318B/27 should have been the only strain remaining in the mixture after the others had been eliminated, but it was not detected when the mixture was tested in the presence of phage 318*b*/27. The result, in this respect, is similar to that obtained for A2 at 30° C.

(*c*) *'Recovery' of a starter after phage attack*

From these findings it seems that in cheese-making practice the acid production by a starter may be largely due to one strain which on daily subculture becomes predominant. If this strain 'fails', the starter will also 'fail', at least temporarily, but it may 'recover', especially if the component strains are unrelated phage types.

Secondary strains arising after lysis by phage, capable of fast acid production, may bring about starter 'recovery' and be able to fulfil the function of the original predominant strain in the starter for cheese-making. Bacterial variation can generally be induced by phage action, and some workers, e.g. Arkwright (5), working with Shiga's bacillus, consider the variation produced by phage to be one of its conspicuous effects.

(*d*) *Period of daily subculture required for a strain to overgrow other strains in a starter*

Determination of the period of daily subculture needed for a 'fast' strain to overgrow the other strains in a starter mixture would indicate how quickly a commercial starter mixture might become virtually a single-strain starter in the factory. In estimating this period the results of replications did not always agree. The conditions and temperatures

of storage undoubtedly cause changes in the behaviour of the strains. When mixtures A and C were originally prepared, 1P5 was predominant in mixture C, 7 days after it had first been prepared, and in mixture A 14 days after the strains had been mixed. The experimental starters were retested 6 or 7 days after mixing and again after 13 or 14 days, and strain 1P5 may perhaps have been predominant earlier. New starter mixtures from the same constituent strains as mixtures A and B were prepared so that 'activity' tests might be made at daily or short intervals.

Three new mixtures of A, namely, A′, A″, and A‴, were prepared. The strains used for A′ had been subcultured daily and incubated at 30° C., whilst those for A″ had been 'rejuvenated' after storage in the ice chest and thus A″ was more comparable with the

Table 6. *Period of daily subculture required for a strain to overgrow other strains in a starter mixture*

Results given in Tables 6 and 7 are similar to those in Tables 3–5, and each figure again represents the average of two obtained from different strengths of inocula, i.e. 0·25 and 0·1%, but the starters were propagated at 30° C. only.

Mixture no.	Days after mixing	Control	Phages added: 1*p*5	144*f*	318*b*/27	Incubation period (hr.)
A′	0	5·1	*4·8*	4·3	4·6	7⅛
	1	5·2	5·2	4·1	5·7	7½
	2	5·9	5·5	5·2	*5·6*	7¼
	3	5·5	4·3	5·1	4·1	7⅛
	4	5·8	4·3	5·2	5·1	7½
	7	5·6	3·9	*5·3*	5·5	7
	9	5·1	2·7	4·9	*4·8*	7⅜
	16	4·6	2·1	4·8	4·7	7⅜
A″	0	4·7	3·7	3·9	4·3	6⅝
	1	4·4	3·5	4·0	4·3	6¾
	2	4·5	3·5	4·0	4·4	6
	3	4·3	3·0	*4·1*	4·3	6¾
	4	4·4	3·2	*4·2*	*4·1*	6⅝
	7	4·6	3·0	4·2	4·6	7
	14	4·7	2·5	4·3	4·6	6⅞
A‴ 1P5—added 1 part 1P5 to 50 parts each of 144F and 318B/27	7	4·7	3·4	4·1	4·6	6⅞

Mixture no.	Days after mixing	Control	Phages added: *l*314/1	144*f*	318*b*/27	Incubation period (hr.)
B″	0	5·6	5·5	4·6	4·9	7½
	1	5·7	5·6	4·8	*5·5*	6½
	2	5·8	*5·5*	4·5	5·1	7
	3	5·5	*5·3*	4·1	*5·3*	7
	4	5·3	4·7	3·9	4·0	7⅝
	7	5·3	*5·0*	3·9	4·6	6½

original A than was A′. In preparing starter A‴ the proportion by volume of strains 1P5, 144F and 318B/27 was altered to 1 : 50 : 50. This mixture was subcultured daily for 1 week before it was tested, and since A‴ differs from the other mixtures it is discussed under (*f*). Starter B″ consisted of the 'rejuvenated' constituent strains of mixture B.

Within 7 days, in the freshly prepared mixtures A′ and A″ (Table 6), 1P5 was largely responsible for the acid produced, and by the ninth day in A′ it was almost entirely responsible. Strain 1P5 appears to take from 6 to 9 days to overgrow the other strains. In mixture B″ 144F was the main contributor to the acid produced by the starter after 7 days' propagation at 30° C., and again 144F predominated after an even shorter period of daily subculturing at 22° C. in A1′ (Table 3D).

In starter mixtures prepared from equal proportions of three strains, one strain may be expected to become responsible for most of the acid produced by the mixture after it has been subcultured daily for 1 week. Thus, the advantages of a multiple-strain starter in affording protection against starter 'failure' due to phage attack during the day's cheese-making may be lost, unless the constituent strains are frequently remixed. The rate at which one strain overgrows the other strains in a starter will probably vary considerably. Strains 1P5 and 144F produce acid and grow very rapidly and consequently may gain predominance in starter mixtures more quickly than the average strain.

Table 7. *Examination of residual strains in a lysed multiple-strain starter for the 'carry over' of phage previously added to eliminate a constituent strain*

Mixture no.	Strain reinoculated into mixture	Days after mixing	Control	Phages added			Incubation period (hr.)
				1p5	*144f*	*318b/27*	
A1′	1P5—added 1 part 1P5 to 2 parts A1	0	4·7	4·7	4·1	4·2	$6\frac{3}{4}$
		1	4·3	3·9	*4·0*	*4·0*	$6\frac{7}{8}$
		2	4·9	3·9	*4·5*	4·3	7
		3	4·2	3·3	4·1	*3·9*	$6\frac{7}{8}$
		4	4·5	3·5	4·4	*4·2*	$6\frac{5}{8}$
		7	4·7	3·4	4·6	*4·4*	$6\frac{3}{4}$
		14	4·7	3·0	4·6	*4·4*	$6\frac{3}{4}$
A1″	1P5—added 1 part 1P5 to 100 parts A1	7	4·9	3·4	4·8	*4·7*	$6\frac{3}{4}$
				Phages added			
				*l*314/1	*144f*	*318b/27*	
B1′	144F—added 1 part 144F to 2 parts B1	0	4·7	4·6	4·0	3·9	7
		1	4·5	4·4	3·8	3·7	$7\frac{1}{8}$
		2	4·4	4·4	3·9	3·6	$7\frac{1}{8}$
		3	4·7	4·8	3·8	3·9	—
		4	4·9	4·4	4·1	3·7	7
		7	4·7	4·9	4·1	3·6	$7\frac{5}{8}$
		14	4·7	4·8	4·2	3·6	$7\frac{5}{8}$
B1″	144F—added 1 part 144F to 100 parts B1	7	4·4	4·6	*4·2*	2·9	$7\frac{1}{8}$

(*e*) *Examination of residual strains of a lysed multiple-strain starter for 'phage carrier' phenomenon*

It is known that lysogenic strains may, during their growth, liberate phage active against other strains (6, 7) and also that some organisms may firmly adsorb phage (8). Experiments were made to determine whether a phage, previously added to a starter in order to exclude the predominant strain, might be 'carried' by the residual strains in a mixture, and later react with the eliminated strain if reintroduced into the starter.

One part by volume of strain 1P5, which had been eliminated from A by phage action, was reinoculated first into two parts of starter A1 to form starter A1′ and then into 100 parts of A1 to give A1″. Similarly, reinoculations were made with strain 144F to give mixtures B1′ and B1″. The new mixtures were tested for 'activity' in the presence of the phages of the constituent strains.

On reinoculation into mixtures A1 and B1 strains 1P5 and 144F immediately affected the acidity produced by the mixtures (Table 7, A1′ and B1′). It appears that the phages of 1P5 and 144F had not been 'carried' by the other strains originally in the starter. Had these phages entered into any symbiotic or similar relationship with the other

strains, the presence of strains 1P5 and 144F could not have been traced so readily on reintroduction into A1 and B1.

(*f*) *Effect of size of inoculum of the constituent strains in the original starter on which strain finally predominates*

In mixture A‴ (Table 6), although the original inoculum of 1P5 was only one-hundredth the volume of strains 144F and 318B/27 together, 1P5 appeared after 1 week to be responsible for most of the acidity produced. When the inoculum of 1P5 was again equal to one-hundredth the volume of A1 in mixture A1″ (Table 7), it also became predominant. Similarly, when the same small quantity of 144F was mixed with B1 to form B1″, it appeared, after 7 days, to be responsible for part of the acidity of mixture B1″.

The proportion in which the strains were mixed to form a multiple-strain starter had, therefore, little influence in determining the final dominant strain. Hence any differences between the strains at the time of mixing in respect of the number of organisms per ml. of inoculum would have little significance in determining the predominant strain.

IV. BEHAVIOUR OF STRAINS USED IN A STARTER MIXTURE DURING CHEESE-MAKING

By preparing starters similar to those already described, in which strains, attacked by different phages, were mixed, the relative contribution of the strains to acid formation during cheese-making could be assessed. The strains obtained by plating samples taken at different stages of making could be identified by their susceptibility to lysis by the phages corresponding to the original strains.

The starter mixtures A and B were again prepared by mixing equal volumes of 18 hr. cultures of each of the three strains together and were used to make cheese immediately after preparation. Cheddar cheese I, IIIA and IIIB were made using experimental starter A and cheese II using B mixture. Cheese IIIA and IIIB were made on the same day, under similar conditions except that they were made at places 4 miles apart by different people. To the milk for cheese IIIB phage 1*p*5 (concentration of particles 10^7 per ml.) prepared in yeast-dextrose broth was added at the beginning of the process, giving a concentration of about 500 phage particles per ml. of milk.

The milk to be used for cheese-making had been previously pasteurized, cooled to 50° F. and, unless made into cheese immediately, stored at a low temperature. The milk was of good bacteriological quality, and on testing was found not to be inhibitory (9, 10). 'Activity' tests were carried out on the strains and on the mixture when it was prepared, using the corresponding phages. Each phage, when added to the mixture, had a significant, if not marked, effect on the acid production, and when added to the corresponding strain almost entirely arrested acid production.

'Activity' tests on the starter mixture and on each component strain were made after adding whey sampled at different stages of cheese-making. Acid production of neither starters nor strains was affected by whey samples from cheeses I, II and IIIA, but the whey from cheese IIIB, though having no effect on the growth of strains 144F and 318B/27, completely suppressed strain 1P5 and slightly retarded that of the mixture.

The following samples were taken during cheese-making and plated on yeast-dextrose agar:

(1) Starter strains.
(2) Experimental starters immediately after mixing.
(3) Cheese-making milk immediately after addition of starter.
(4) Mixtures of curd and whey after cutting the curd.
(5) Curd and whey separately at the time of drawing the whey.
(6) Cheese after being in the press for 24 hr.

The preparation of the first dilutions of the curd or cheese samples was facilitated by shaking with glass beads.

Counts were made after incubation for 2 days at 30° C. Between ten and twenty colonies were selected (11) for identification by phage lysis using the broth test (1), and consequently the presence or relative numbers of any strain could be traced as cheese-making proceeded.

Table 8. *Approximate percentage of different starter strains obtained at different stages of cheese-making (strains identified by phage lysis)*

Cheese no. ...	I			II			III A Control			III B + phage 1*p*5		
Starter used ...	Mixture A			Mixture B			Mixture A			Mixture A		
Strains ...	1P5	144F	318B/27	L314/1	144F	318B/27	1P5	144F	318B/27	1P5	144F	318B/27
Starter at time of mixing strains ...	80	15	5	25	30	45	50	10	40	50	10	40
Time of sampling:												
Adding starter	50	10	40	60	20	20	27	40	33	40	27	33
Cutting	40	40	20	47	20	33	7	53	40	20	40	40
Drawing whey:												
Curd	40	30	30	54	13	33	33	40	27	0	40	60
Whey	62	13	25	13	40	47	40	27	33	0	73	27
Milling:												
Curd	78	11	11	33	20	47	74	13	13	0	47	53
Whey	70	20	10	—	—	—	73	7	20	0	60	40
Pressing (24 hr.)	90	0	10	—	—	—	54	33	13	0	87	13
Relative no. of organisms present per ml. of milk culture, obtained by plating strains immediately before making starter mixtures	50	12	38	27	13	60	75	5	20	75	5	20

The proportion of the three different strains in the original starter and in the samples taken at different stages of cheese-making will be seen in Table 8. The relative number of organisms present in the cultures of the different strains used to form the starters was computed from the plate counts and is also given. Strain 1P5 was present in greater numbers than the other strains in the original starter mixture and in the milk at the beginning of cheese-making. It continued to be the predominant strain throughout cheese manufacture (I and III A). In experimental cheese II the results are less consistent, strain 318B/27 predominated in the starter mixture and towards the end of cheese-making, but strain L314/1 seemed to predominate from the addition of the starter to the milk until the whey was drawn.

When phage 1*p*5 had been added to the vat (cheese III B) strain 1P5 could not be detected at a stage later than cutting. Although acid production was retarded at the beginning of cheese-making, towards the end it was not noticeably 'slower' than the control (III A); the remaining strains in the starter mixture quickly masked the 'failure'

of 1P5 and the starter 'recovered'. Had strain 1P5 been used alone as starter and been attacked by a phage of similar potency, a 'slow' or 'pack up' cheese would have resulted.

SUMMARY AND CONCLUSIONS

1. Three experimental starters were prepared by mixing three unrelated phaging strains of *Str. cremoris*. The corresponding phages were used to determine whether changes occurred in the relative numbers of these strains on daily subculture.

The results indicated that (*a*) in a multiple-strain starter one strain may overgrow the others and become responsible for most of the acid produced by that starter about 1 week after mixing the strains. The starter may soon become virtually a single-strain starter. (*b*) Even during the day's making, one of three strains, mixed together at the time of introduction into the cheese vat, may partly overgrow the other two strains. (*c*) The dominance of a particular strain in a starter mixture may be affected by the temperature of propagation.

2. After a strain had become predominant in each starter mixture, it was eliminated by the corresponding phage and one of the residual strain usually increased in 'activity' and restored the acid-producing properties of the starter. In a cheese-making experiment, when one strain was removed by phage lysis from a multiple-strain starter, the acidity in the vat was temporarily retarded but the starter 'recovered' owing to the increase in acid production of the other strains. In commercial cheese-making the mode of starter 'recovery' after phaging has occurred is probably often similar, although natural variants and secondary phage-resistant strains may contribute. The results indicate certain merits and limitations of multiple-strain starters.

3. After the elimination of the predominant strain from a starter by phage the strain was reinoculated into the experimental starter and quickly affected acid production by the starter.

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356. BACTERIOPHAGE IN CHEESE MANUFACTURE

CONTAMINATION FROM FARM EQUIPMENT

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It is now generally recognized that 'slowness' in cheese manufacture is commonly due to destruction of the acid-producing starter streptococci in the milk and curd by bacteriophage. Methods for preventing the phage infection in commercial practice are still being worked out, and general agreement has not yet been reached among workers in this field although basic principles are well established. Apparently the tendency in England (1, 2) is to use starters containing a mixture of several strains of streptococci (mixed starters) and, guided by a 'phage-development test' on the cheese whey, to change the starter in use as soon as a significant concentration of phage for the starter appears in the cheese vats. In New Zealand, on the other hand, the tendency is towards the use of 'single-strain' starters because of their greater and more regular activity and their capacity to produce a cheese of closer texture. Wherever one of the few stock single-strain cultures is in use, phage for the starter is constantly present in the cheese whey, but experience has shown that this has no untoward effect provided that the concentration is kept below a certain level, by maintaining the starter itself free from phage and by sterilization of the factory and farm utensils. Even where one of the stock cultures has not been used in a given factory for several months the specific phage is present (presumably in dormant form) in the surroundings, and it rarely fails to appear in the whey if not on the first then on the second day after the starter is used again. It seems to us, therefore, that a large number of unrelated mixed starters would be required under the English system if the whey is to be kept entirely free from phage. It will be interesting to see whether the system can be maintained on its present basis in England over several cheese-making seasons.

Where a starter has been in use in a cheese factory for a lengthy period there is invariably a low and harmless concentration of phage daily in the whey, which is extremely difficult to eliminate. It is probably due to air-borne contamination of the milk while the vats are being filled. Apart from this there are three possible sources for the dangerously high concentrations which sometimes occur:

(i) The starter culture itself.

(ii) The surfaces of factory utensils and equipment.

(iii) The milk delivered to the factory.

The first two can be dealt with by isolation and protection of the starter (3) and by sterilization of equipment by heat or disinfectants. The third source exists in New Zealand dairy practice because farmers collect cheese whey from the factories in the cans in which they deliver the milk and sometimes fail to sterilize the cans completely before they are used again for milk. If special arrangements had to be made for the transport of whey back to farms, the pig-rearing industry might be rendered uneconomic. Farmers who deliver only a small amount of milk are able to collect whey in special

vessels on their carts or trucks, but suppliers of larger amounts of milk cannot accommodate both milk cans and whey vessels on the one vehicle. Thus they must either use their milk cans to carry whey or incur the expense of a special trip back to the factory. It seems, therefore, that in New Zealand the carriage of whey in milk cans must be regarded as necessary and a condition to be accepted in any proposed solution of starter problems.

'Slow-vat' losses due to phage in the milk are difficult to assess. The trouble is spasmodic and sometimes so mild that it is not recognized for what it is. But during the last few years it has gradually become clear that traces of phage in cheese milk cause much more trouble than has been realized. Hunter (4) has described the type of effect which may occur. As with many human diseases the incidence appears to increase when once the exact nature of the trouble is understood and diagnosis is more precise. Factory managers who at one time had considered that their vats were 'working' normally have come to realize that there is often a slight phage effect towards the end of the cheese-making process which has a small but definite influence on the quality of the cheese. In some factories there is still no trouble of this kind, whether because the farmers clean and sterilize the milk cans more efficiently or for some other reason is not known. Other factories experience no trouble for long periods and then have an epidemic of it. Still, on the whole, it is to be expected that wherever whey is carried in milk cans phage will be present in the cheese milk from time to time.

In view of the position which was thus gradually disclosed it became necessary to determine whether there are any practicable means whereby either

(i) milk cans may be sterilized effectively after being used for the carriage of whey;
or (ii) phage present in whey may be destroyed before the whey is collected by farmers;
or (iii) the adverse effect of phage in cheese milk may be circumvented.

The first alternative would perhaps seem the simplest, but in practice it is not so, because it depends upon the actions of a large number of individuals some of whom from time to time may be unreliable. Actually no systematic attempt has been made to apply this method of control because we consider that it is never likely to be wholly successful. The other two alternatives have been explored, and at the present time a method of applying the third is being tested in commercial practice. The present paper gives details of the various trials.

ESTIMATION OF PHAGE CONCENTRATION IN WHEY

For the purpose of following the effect of any treatment of whey on its phage content the following method was used except where otherwise specified. A series of decimal dilutions in water of control or treated whey was prepared. Standard drops (from a platinum loop 2 mm. in diam.) were then placed on a mat of the susceptible organisms previously spread on lactose-yeast-phosphate agar (5). The log of the reciprocal of the highest dilution giving plaques was recorded as the titre of the phage. Thus a titre of 2 means that plaques were observed on the area covered by the 1 in 100 dilution but not the 1 in 1000 dilution of the whey. As Nichols & Wolf (6) have pointed out this titre is not necessarily a true estimate of the phage concentration. Addition of drops of the serial dilutions to cultures in sterile milk almost invariably gives lysis of the organisms with a higher dilution than that which just shows plaques on agar. But the agar-plate method has proved sound for comparative purposes, and it has great advantages in convenience and simplicity.

Phage concentration normally encountered in whey

Where gross infection of a cheese vat with phage from starter, and factory and farm utensils is eliminated, air-borne infection of the cheese milk before rennet is added leads to the development of a low concentration of phage in the whey during the manufacturing process. At the time the whey is drained from the vat the titre ranges from 1 to 4, depending on the precise degree of original infection and the culture in use. The phage continues to increase in concentration during the period in which the whey is stored in a tank outside the factory. On the following morning, when collected by the farmer, the whey may have a phage titre as high as 15. It is thus readily understood how milk cans after contact with this whey may infect the milk.

Destruction of phage in cheese whey by heat

The various phage races which attack lactic streptococci have different individual thermal death-points, but all are destroyed in whey by treatment at 75° C. for 10–30 min. (6, 7). It is difficult to copy in the laboratory the treatment applied in a plate pasteurizer, and thus the exact 'flash' temperature needed to destroy phage is not known. Rough experiments indicate that nothing less than 180° F. would be effective. Most modern cheese factories in New Zealand use a plate-type pasteurizer for the treatment of the milk, and it would therefore be possible to use these machines for heat treatment of the whey after its removal from the cheese vats. Three trials were carried out in a commercial factory to decide whether this was practicable. The whey ran from the vats into a tiled tank sunk below floor level. Normally it was pumped from the tank and delivered to a separator. In these trials it was pumped from the tank, through the plate pasteurizer and then to the separator. This was quite easily arranged with a little extra piping. The whey entering the pasteurizer at about 90° F. was heated to 180–190° F. (no automatic temperature control was fitted) and regeneratively cooled to 90° F.

In the first trial whey from six vats (about 4500 gal.) was treated. The process went quite smoothly, and contrary to expectation the precipitated protein from the pasteurized whey did not cause a blockage in the separator (a machine with a rated capacity of 1100 gal./hr., but evidently being run over its rated capacity). It was not possible under the circumstances to do accurate determinations of phage titres in the whey, but rough tests showed a titre before treatment of 4 and after treatment of nil (i.e. no plaques from undiluted whey). There was, however, a trace of phage left in the whey because a loopful added to a 10 ml. milk culture of the starter in use caused lysis in 24 hr. at 22° C. Possibly the somewhat fluctuating temperature of pasteurization accounted for the incomplete 'kill'. From the mechanical point of view there was no difficulty either with pasteurizer or with separator. A slight 'burning-on' of protein occurred on the pasteurizer plates, but not to a serious degree. Deposit in the separator bowl was bigger in bulk than usual and was granular instead of gelatinous; the separator was consequently more easily cleaned. The second trial gave similar results.

On the third occasion, however, mechanical difficulties were encountered. The whey from nine vats (about 6750 gal.) was treated. When about three-quarters of the whey had passed through the pasteurizer the 'burnt-on' protein had formed so thick a layer on the plates that heat conduction was seriously impaired, and it was impossible to reach

a pasteurizing temperature of more than 175° F. with a normal rate of whey flow. The pasteurizer was very difficult to clean after this trial run. The separator, however, still did not suffer a blockage.

These trials were carried no further in the meantime, since the mechanical difficulties together with the cost in steam (which would be equal approximately to the cost of pasteurizing the milk in the first place) made it desirable to seek a cheaper and more convenient method.

DESTRUCTION OF PHAGE IN WHEY BY CHLORINATION

Hypochlorites are the chemical disinfectants of choice for dealing with phage in the dairy factory. The possibility of destroying phage in cheese whey by chlorination was therefore considered, although it was realized that the presence of protein in whey would result in non-specific fixation of much of the available chlorine. Several compounds with available chlorine ranging from 27 to 65% were obtainable. For large-scale experiments chloride of lime (27–30% available Cl) was cheapest and quite suitable. The powder was mixed with water (1 lb. to 1 gal.) and the insoluble portion was allowed to settle. The supernatant hypochlorite liquor was titrated for available chlorine and used in the appropriate quantity, which when mixed with the whey gave the desired available chlorine concentration.

Preliminary laboratory trials indicated that whereas concentrations of available chlorine of over 200 p.p.m. in whey would inactivate phage completely where its original titre was 2 or 3, lower concentrations of the order of 150 p.p.m. would inhibit further development, possibly by destroying the substrate bacteria. Various trials on a commercial and semi-commercial scale of chlorine treatment at different stages from the cheese vat to the storage tanks were carried out. Results from a few typical trials will be discussed.

ADDITION OF HYPOCHLORITE TO THE CHEESE VAT

After a control whey sample had been taken hypochlorite liquor was added with vigorous stirring to the curd and whey in the cheese vat 5 min. before the whey was run off. Four gallon samples of control and treated whey were held at room temperature for examination at 6 and 24 hr. The results given in Table 1 indicate that 144 p.p.m. of available chlorine is capable of preventing further multiplication although the phage is not destroyed.

Table 1. *Addition of hypochlorite to curd and whey in cheese vat*

Whey sample	Initial conc. Cl in whey (p.p.m.)	Phage titre (log 1/dilution)			
		Before addition	5 min.	6 hr.	24 hr.
Control	Nil	3	—	10	12
Treated	35	—	3	10	10
Control	Nil	3	—	8	10
Treated	72	—	3	5	7
Control	Nil	3	—	10	12
Treated	144	—	3	2	2

It is of interest to record that although a strong smell of chlorine was evident on the curd in the above experiments the resultant cheeses were all of good quality and suffered absolutely no adverse effect from the chlorine treatment.

In one experiment samples of curd and whey as well as samples of whey alone were taken from cheese vats. The results are shown in Table 2. It will be observed that where the cheese curd was present in the mixture the phage reached a high titre, even where the larger amount of hypochlorite was present initially. Probably the continued expulsion of phage-laden moisture from the curd after the hypochlorite is all decomposed accounts for this result.

Table 2. *Hypochlorite action on phage in whey and in cheese-curd + whey*

Sample	Initial conc. Cl in whey (p.p.m.)	Phage titre (log 1/dilution) Before addition	6 hr.	24 hr.
Control whey	Nil	3	10	12
Treated whey	72	—	6	9
Treated whey	144	—	2	3
Control whey + curd	Nil	3	11	11
Treated whey + curd	72	—	11	11
Treated whey + curd	144	—	10	12

Although addition of hypochlorite to the cheese vat just before the removal of the whey would give the result desired the practice would not be permissible in commercial factories, if only because of the effect produced in the whey cream. Concentrations of hypochlorite of the order necessary to inhibit phage gave an objectionable taste in the whey cream and rendered it quite unsuitable for the manufacture of whey butter. The rest of the trials were therefore confined to treatment of the cheese whey as it left the separator. In commercial practice this can be done quite conveniently by having a reservoir fitted with a tap for the hypochlorite liquor near the separator and allowing the liquor to flow at a suitable rate into the hopper which receives the separated whey for pump delivery to the whey tank outside the factory. Under this system the whey is mixed with the hypochlorite very thoroughly before the phage has had time to reach a high titre. Table 3 gives the results of typical experiments on these lines. The results indicate that 180 p.p.m.

Table 3. *Addition of hypochlorite to cheese whey at whey separator*

	Initial conc. Cl in whey (p.p.m.)	Phage titre (log 1/dilution) Before addition	Immediately after addition	24 hr.
Phage race hp	180	3	3	0
	180	2	2	0
	85	3	3	8
Phage race r_1	180	7	7	3
	130	6	7	5
	85	6	6	8

available chlorine in whey as it leaves the separator causes an actual decrease in the titre during 24 hr., whereas 85 p.p.m. fails to prevent entirely the multiplication of phage. The higher initial titres with r_1 are characteristic of this phage which develops more readily under the conditions obtaining in the cheese vat.

Addition of hypochlorite to whey containing low and high titres of phage

As a matter of interest one further experiment was carried out to determine the effect of chlorine on phage allowed to develop in whey to a high titre as compared with the same phage at a low titre—the results are set out in Table 4. The results given in Tables 3 and 4 considered together indicate that 150 p.p.m. of chlorine is the lowest concentration

which will cause a reduction in phage titre during a 24 hr. storage period. Over 200 p.p.m. is necessary to destroy low-titre phage completely (as judged by the plate method), while high-titre phage requires treatment with over 500 p.p.m. available chlorine for its destruction. This applies to phage race hp. Other experiments indicated that r_1 phage at high titre required even higher concentrations than 500 p.p.m. In a few trials the treated whey was tested for phage by examination for lytic action in liquid media instead of by the plate method. As was anticipated it was found that over 600 p.p.m. available chlorine was needed to eliminate phage from high-titre whey when destruction was judged by the more delicate lytic test.

Table 4. *Effect of hypochlorite on low- and high-titre phage in whey*

Initial conc. Cl in whey (p.p.m.)	Phage titre (log 1/dilution)		
	Before addition	6 hr.	24 hr.
	Low-titre phage hp		
Nil	4	12	15
36	—	9	9
72	—	7	8
144	—	2	2
180	—	1	3
216	—	0	Nil
252	—	0	Nil
288	—	0	Nil
576	—	Nil	Nil
	High-titre phage hp		
Nil	12	—	12
144	—	—	6
216	—	—	4
252	—	—	3
288	—	—	2
432	—	—	0
486	—	—	0
648	—	—	Nil

Note. 'Nil' means that no plaques were given by the undiluted whey

PIG-FEEDING TRIAL

If bleaching powder were to be used in cheese whey as a means for destroying or limiting the development of streptococcal phages the question of the suitability of the treated whey as food for pigs arises. A trial was therefore made on the following lines.

Two separate groups of young pigs were selected, one from litters averaging 50–70 lb. live weight and the other from litters averaging 80–120 lb. Each group was sorted into two lots A and B in pairs similar in sex and breeding. One lot A of each group was fed on whey, meat and bone and barley meal; the other lot B was fed on chlorine-treated whey, meat and bone and barley meal. Each lot comprised five pigs which were all fed similar amounts of whey and meal.

At the start of the trial the chlorine-treated whey contained bleaching powder at the rate of 3 g./gal. (=180 p.p.m. available chlorine). Increases were gradually made until the rate was trebled towards the end of the feeding period (9 weeks). The strong smell of chlorine present in the whey immediately after the addition of hypochlorite liquor almost disappeared during overnight storage. The chlorination treatment evidently destroyed many bacteria as well as phage, since the treated whey retained its original 'sweet' flavour in contrast with the untreated whey which frequently had a 'yeasty' or 'fermented' flavour. The pigs fed on the chlorine-treated whey suffered no ill effects whatever. There

was no difference either in growth rate between experimental and control animals or in the quality of the bacon produced.

There is thus no reason why cheese whey should not be chlorinated for the limitation of phage development unless the cost is considered prohibitive. With chloride of lime at 6*d.* per lb. treatment of the whey from a 700 gal. vat at the rate of 3 g./gal. would cost 2*s.* 4*d.* As has already been mentioned, the question of whether such a regular daily expenditure per vat is justifiable depends on the amount of cheese spoilage which is caused by the presence of phage in the cheese milk supplied.

Use of starters in rotation

While the trials of heat treatment and chemical disinfection of cheese whey were proceeding the idea of using a system which takes advantage of strain specificity in streptococcal phages was being considered. It is evident that, providing a series of single-strain starter cultures is so selected that no two of them are attacked by a given phage, an appropriate choice of the starter for use in cheese vats on any day will ensure that any phage likely to be present in the cheese milk cannot attack the starter. The idea is attractive because the system is costless, unlike the other systems which involve destruction of the phage present in whey. Mr J. Thompson, of the Featherston Co-operative Dairy Co., was the first manager to try this scheme in commercial practice. Under New Zealand conditions the whey run from the vats on a given day is taken home by the farmers next day, and traces are liable to be present in the milk delivered to the factory on the third day with sometimes a slight carry-over to the fourth day (due to some of the later whey draining from the vats being held over a day in the factory). The essence of the choice of starters lies therefore in the use of a different culture each third and fourth day. For example, four independent single strains would be used as given in Table 5.

Table 5. *Rotational use of single-strain starters*

	Mon.	Tues.	Wed.	Thurs.	Fri.	Sat.	Sun.	Mon.
Starter in use in vats	HP	R_1	R_6	E_8	HP	R_1	R_6	E_8
Phages possibly present in milk	r_6, r_1	e_8, r_6	hp, e_8	r_1, hp	r_6, r_1	e_8, r_6	hp, e_8	r_1, hp

The early trials of this rotational system soon showed that it was successful in overcoming the bulk of the trouble caused by whey contamination of the milk cans. The use of the system has gradually spread until at the present time it is undergoing trial on a wide commercial scale.

The system has advantages other than that of overcoming starter failures due to phage in the cheese milk. Since a different culture is in use as a starter each day, phage contamination of the milk from the factory equipment and air-borne phage as a source of contamination for the bulk starter both have considerably less significance than when the same starter is used every day. The danger from phage infection carried over from day to day does not disappear entirely, however, since it has been shown that phage persists in the surroundings for months at least; but the danger is obviously infinitely less.

On the other hand, adoption of the system involves two problems, one for the bacteriologist and one for the cheese-maker.

(*a*) It is necessary to isolate a sufficient number of *active* strains of streptococci (four at least but more if possible) which are quite independent in their phage reactions. Experience has shown that after three such cultures have been found it is difficult to isolate further strains which are both *very active in acid production* and quite independent from the first three in phage reaction. We have now six independent cultures, but three of them are not quite as active as the cheese-maker would desire, although, provided an increased percentage of culture is used in the cheese vat, they enable excellent cheese to be made. The isolation of further suitable cultures becomes progressively more difficult because each possible culture tested must be unaffected by all the phages, for the cultures already in use and its own appropriate phage must not effect the other cultures.

(*b*) A disadvantage of the system which cannot be overcome except by the skill of the cheese-maker is the slightly different acid-producing characteristics of the cultures. When the system was first proposed we were of the opinion that this drawback would be fatal to its adoption in commercial practice. Cheese-factory managers report, however, that with experience they can adjust their cheese-making practice from day to day to allow for this difference between the cultures, and thus produce cheese of consistent quality. This adjustment is more easy to make under our rotational scheme than it is under the English scheme (1, 2) because our cultures have constant characteristics which the manager soon learns to depend upon, whereas under the English scheme (if we understand it correctly) fresh mixed starters of unknown characteristics are constantly being introduced. Nevertheless, the slight differences between our several cultures are undoubtedly a drawback to the rotational system. In the course of time sufficient independent cultures of almost identical activity may be isolated; otherwise the only way to get complete regularity of acid development from day to day is to apply one of the methods of destroying phage in the whey and thus permit the constant use of one single strain starter culture.

SUMMARY

Bacteriophage infection of the starter streptococci, which commonly causes 'slowness' in cheese manufacture, may arise from whey infection of milk cans used for the cartage of cheese whey from factory to farm. Thus 'slowness' can occur even when the starter itself and all the factory utensils are free from infection. The pasteurizing treatment which the cheese milk receives is not sufficient to destroy the traces of phage derived from infection of the milk cans.

Three methods for eliminating this trouble were investigated:

(i) Pasteurization of the whey at 190° F. for a few seconds immediately after removal from the cheese vat.

(ii) Treatment of the whey with chloride of lime.

(iii) The use of a series of single-strain starters in daily rotation in the cheese factory.

All three methods proved practicable, although some mechanical difficulties were encountered in the particular pasteurization system which was tried. The third method is at present undergoing an extensive trial on a commercial scale. It is preferred in the first place because it is costless, whereas the first two methods would involve a regular additional cost in steam or chloride of lime.

Our thanks are due to Messrs A. Fraser and F. Schwieters of the Joll Dairy Co. for facilities provided and for assistance in the commercial scale trials.

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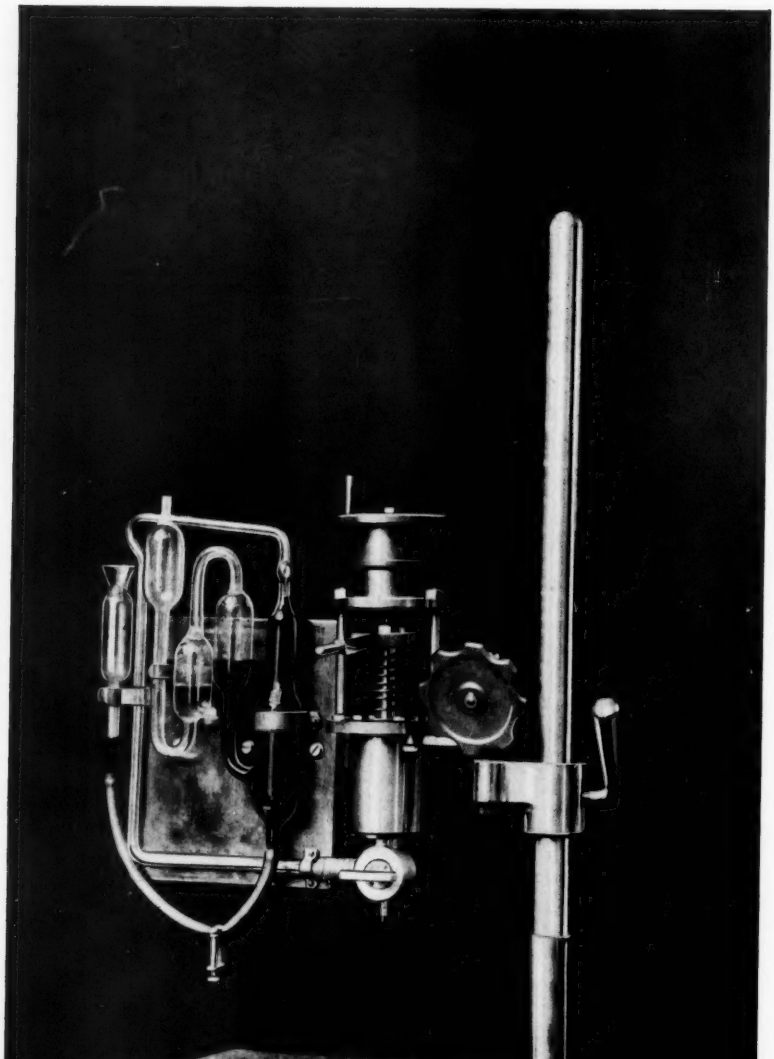

THIEL AND OTHERS—ESTIMATION OF OXYGEN IN MILK POWDER

637.143:545.721

357. APPARATUS FOR ROUTINE ESTIMATION OF OXYGEN IN TINS OF MILK POWDER

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(With 1 Plate and 2 Text-figures)

Introduction

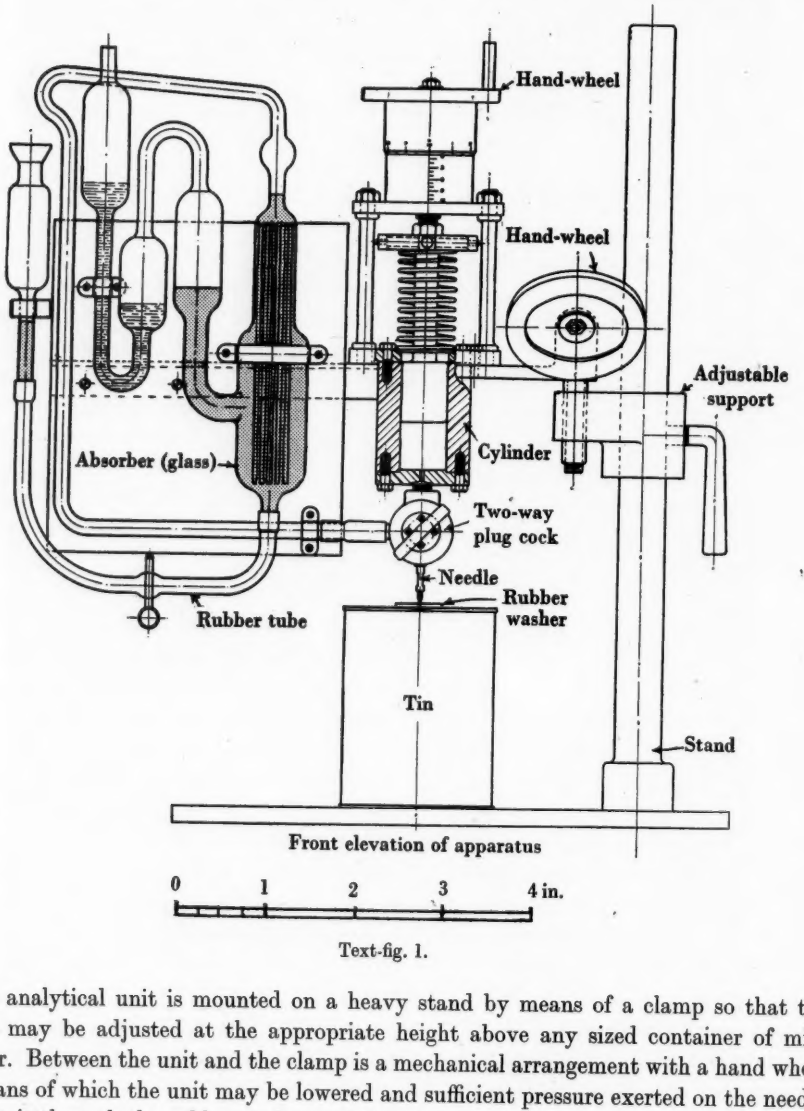
For the routine estimation of oxygen in the headspace gas of tins of dried milk conventional gas analysis apparatus has several disadvantages. Slowness of operation and fragility of all-glass apparatus are the chief disabilities of methods previously described in the literature, except for that described by Strachan & Embree(1). Their method, however, requires 70 ml. of gas for each determination which is more than can be conveniently withdrawn from tins containing only 12 oz. of powder. The method described in this paper is applicable to all sizes of tins and is suitable for routine use in factories in respect to degree of manipulative skill required and speed of operation. The apparatus has proved highly satisfactory in factory use for the last year. It may be dismantled and cleaned in 15 min. so that maintenance time is cut to a minimum; the device for sampling from all sizes of tins is an integral part of the apparatus which is all metal except for the absorption bulbs; and twenty samples can without undue effort be taken and analysed in an hour.

Description of apparatus

In principle the method is conventional, the gas volume being measured at constant temperature and pressure before and after absorption of oxygen, but the apparatus employed differs in several respects from similar devices previously described. Instead of a glass pipette with confining fluid for volume measurement, a metal piston and cylinder are used. The position of the piston in the cylinder, and hence the volume of confined gas, is accurately estimated by reference to a micrometer screw device which actuates the piston against the thrust of a return spring. The micrometer head is divided into 100 parts, and 1 complete revolution alters the volume of the cylinder by about 1 ml. It is not, of course, necessary to know the absolute volumes of the unknown gas mixture before and after absorption but only the relative volumes, and these are accurately given by the micrometer screw device which controls the position of the piston in the cylinder. The piston may also be moved by a hand lever, and this is used to force the confined gas rapidly in and out of the absorption pipette. A volume of gas equivalent to 10 revolutions of the divided head is taken for each analysis, so that taking into account the position of the decimal point the percentage oxygen in the mixture is given directly by the contraction in volume after absorption.

The sampling device is an integral part of a two-way cock connecting the gas cylinder to the absorption pipette. The cock is attached to the bottom of the cylinder and there is a side lead to the absorption pipette. A special needle screws into the underside of the barrel of the cock. The general arrangement may be seen in the photograph in Pl. 1 and

the sectional drawing in Text-fig. 1.* Some difficulty was experienced in designing a suitable needle in which the orifice remained free from particles of rubber, but the design illustrated in Text-fig. 2 is satisfactory. A test needle assembly made from Firth-Vickers cutlery grade stainless steel (FH), quenched in oil from 960° C. and tempered at 180° C., showed no sign of wear or tendency to clog after piercing 2000 holes.



Text-fig. 1.

The analytical unit is mounted on a heavy stand by means of a clamp so that the needle may be adjusted at the appropriate height above any sized container of milk powder. Between the unit and the clamp is a mechanical arrangement with a hand wheel by means of which the unit may be lowered and sufficient pressure exerted on the needle to force it through the rubber gasket and then through the tin.

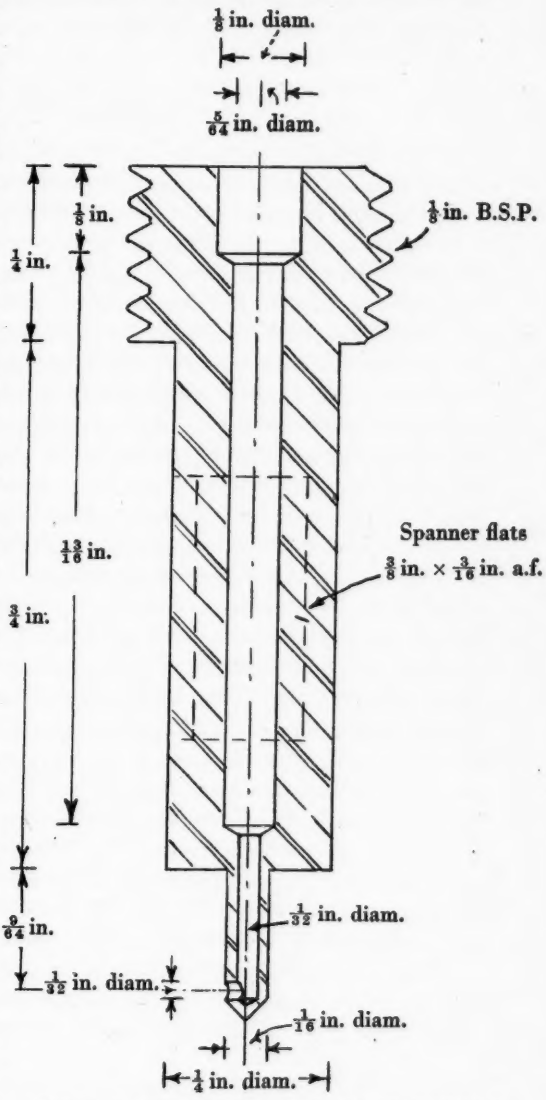
* Complete working drawings of the apparatus may be obtained on application to the Secretary, Council for Scientific and Industrial Research, 314 Albert Street, Melbourne, Australia, or Officer in Charge, Engineering Department, National Institute for Research in Dairying, Shinfield, near Reading, England.

The cylinder, end-plates and barrel of the tap are of 18/8 stainless steel, the piston of hard-chrome plated mild steel and the plug of the tap of nickel silver. The piston and cylinder should be ground to a high quality finish concentric and parallel so that with a clearance of no more than 0·0003 in. the piston will move freely in the cylinder. The male and female threads of the micrometer should be cut on a high-quality precision lathe. The stainless steel needle screws into the body of the cock against a silver gasket. The rest of the apparatus is of mild steel and brass, chromium plated.

The metals used in the apparatus were chosen to avoid corrosion and seizure. After a year's use under factory conditions no sign of corrosion has been detected and the piston and the plug of the tap have always moved freely.

The reagent used with this apparatus is *M* chromous sulphate in *N* sulphuric acid, and as the reagent is acid a preliminary absorption of carbon dioxide is not necessary. The reagent is made by dissolving 66 g. $Cr_2(SO_4)_3.15H_2O$ in water containing 10 ml. concentrated sulphuric acid and making to 200 ml. This solution is then reduced by shaking in a bottle with about 20 g. of granulated zinc dissolved in 100 ml. of mercury. Complete reduction, indicated by a sky blue colour, is obtained in 10–15 min. (2). The protecting bulbs are filled with *M* ammonium chloride solution.

The absorption pipette is connected to the side arm of the two-way cock by a long capillary lead and just above the reference mark it expands into a safety bulb. The relatively large dead-space in the connexion greatly increases the number of times the gas must be transferred from the gas cylinder to the absorber to ensure complete absorption of oxygen, but decreasing the volume of the deadspace introduces manipulative difficulties. When a sample of gas is drawn into the gas cylinder from a tin of milk powder the pressure of the gas may be slightly above or below atmospheric. Thus on turning the two-way cock to connect the absorption pipette to the gas cylinder a surge of gas may



Text-fig. 2. Sampling needle. (Material—cutlery stainless (Firth-Vickers F.H.)).

occur either into or out of the cylinder. If the gas in the cylinder is under reduced pressure, the safety bulb will prevent reagent from being drawn into the metal parts of the apparatus; if the gas is above atmospheric pressure, some mixing of the sample and inert gas in the connexion will occur, but in our experience oxygen in the sample will not reach the chromous sulphate solution before the volume of the sample has been measured.

Method of determining headspace oxygen

After the apparatus has been assembled, freed from oxygen, and tested for leaks as described below, a determination of headspace oxygen is made in the following way:

On the tin to be tested place a red rubber gasket about $1\frac{1}{2}$ in. diameter by $\frac{1}{4}$ in. thick to the top side of which a $\frac{1}{8}$ in. thick rubber washer has been cemented. Fill the trough formed by the washer with water and lower the needle of the instrument into it. Then adjust the meniscus in the absorber to the reference mark and turn the cock 45° anti-clockwise. Turn the divided head 1 revolution clockwise to ensure a positive pressure in the cylinder and turn the tap to bring the needle and cylinder into communication. Rotate the divided head as far as possible in a clockwise direction thus forcing the excess inert gas to waste through the water seal. Lower the needle by means of the hand wheel until the gasket and then the tin is pierced, then rotate the divided head anti-clockwise until the reading is 10·000 and turn the cock clockwise through 45°. Rotate the divided head about $\frac{1}{6}$ revolution clockwise so that the pressure in the cylinder will probably be above rather than below atmospheric and then turn the tap very carefully to bring the absorber and cylinder into communication.

Adjust the meniscus in the absorption pipette to the reference mark and read the gas volume on the micrometer. Turn the head $2\frac{1}{2}$ revolutions clockwise and then move the piston up and down 30 times in approximately 45 sec. by means of the hand lever. The faster the lever is moved the more rapidly will oxygen be absorbed, but if it is moved too rapidly bubbles form in the chromous sulphate solution and these are likely to be carried from one interface to the other in the absorption pipette. Bring the liquid level on to the reference mark again and read the gas volume. Absorption may then be continued for a few strokes to check whether all the oxygen has been absorbed. Consecutive readings should agree to $\pm 0{\cdot}002$ revolution. The difference between the final and original readings multiplied by 10 gives the percentage of oxygen in the gas sample. If the original pressure in the tin is considerably different from atmospheric, it may be considered necessary to take account of the initial volume of the sample in calculating the percentage of oxygen.

Better reproducibility of results is obtained if the meniscus is always brought on to the reference mark from above. In this way errors due to slackness of the micrometer screw in the thrust plate are avoided.

Assembling and testing the apparatus

Every 2 or 3 days the apparatus should be dismantled, cleaned and reassembled. The plug of the cock is first removed and next the micrometer head by unscrewing the nuts at the top ends of the pillars. The piston may then be withdrawn and all bearing surfaces cleaned with a soft cloth. Great care should be taken in handling the piston and the plug of the cock as the slightest damage to the ground surfaces may render the apparatus useless.

While the cock is dismantled the level of chromous sulphate solution in the absorption pipette should be adjusted to the reference mark. This may be done by adjusting the quantity of *M* ammonium chloride in the protecting bulbs. If necessary the chromous sulphate solution should be renewed at this stage. We have found that 50–100 determinations may be made before the rate of oxygen absorption falls appreciably, and it is convenient to have at hand a charge of reduced chromium solution stored over zinc amalgam so that the solution in the pipette may be renewed at the beginning of the day if a large number of determinations is contemplated.

The cock is assembled using a minimum of heavy steam cylinder oil (600W, Vacuum Oil Company) as lubricant. It is important to push the plug well home to force out any excess oil. Smear the piston with a small amount of light mineral oil (S.A.E. 30) and very carefully slide it with a rotary motion into the cylinder. A few drops of S.A.E. 30 oil may with advantage be placed on the top of the piston. Lubricate the bearing at the top of the piston rod with light oil and reassemble the micrometer head.

So that a constant water vapour tension may be maintained in the gas space a few mg. of water should be present in the cylinder. Remove the needle and draw in about $\frac{1}{2}$ ml. of water. The excess may then be blown out by repeatedly raising and lowering the piston with the hand lever, after which the female thread in the underside of the cock should be dried with a cloth and the needle replaced after renewing the cotton-wool plug in the counterbore.

The assembled apparatus should then be freed from oxygen and tested for leaks. Take in a full charge of air and absorb the oxygen as already described. Turn the tap 45° anti-clockwise and raise about 5 lb./sq.in. positive pressure in the cylinder by turning the divided head 2 revolutions clockwise. After about 1 min. release this pressure by turning the micrometer head to its original position, turn the cock to the absorbing position and adjust the meniscus. If no leak has occurred, the micrometer reading should be within $\pm 0{\cdot}002$ revolution of the original reading. If there is a reduction in gas volume, the plug of the tap has probably not been pushed home.

STANDARDIZATION

When surplus inert gas is blown off to air before a fresh gas sample is taken, a small amount remains in the bore of the tap and needle. On taking in the next sample this residual inert gas returns to the cylinder and must be allowed for by setting the micrometer to a negative reading when the piston is at the bottom of its stroke. This adjustment is made by loosening the nut on the top of the micrometer, rotating the divided head to the estimated reading, and then locking it in position by tightening the nut. By making a series of analyses of air and assuming an oxygen content of 21·00% in the atmosphere it was found by calculation that the micrometer on the original instrument should be set at $-0{\cdot}250$ revolution when the piston was in contact with the end of the cylinder.

The following example illustrates the type of calculation involved. With the micrometer set at zero when the piston was in contact with the bottom of the cylinder, 10·052 'revolutions' of gas (air + the residual inert gas) were drawn into the cylinder. After complete absorption of oxygen the micrometer read 7·993 so that the oxygen in the sample was equivalent to 2·059 revolutions which would be contained in $(2{\cdot}059 \times 100)/21 = 9{\cdot}804$ revolutions of air. Therefore the quantity of inert gas that returned to the cylinder was equivalent to $10{\cdot}052 - 9{\cdot}804 = 0{\cdot}248$ revolution.

Discussion

A series of analyses was made of mixtures of nitrogen and oxygen containing approximately 0·5, 2, 3 and 5% oxygen to determine the reproducibility of results. The compositions of the mixtures were also determined with a Haldane gas analysis apparatus. Replicate determinations usually agreed to ±0·02%, but occasionally differences were as great as 0·1%. These occasional discrepancies were probably due to transfer of bubbles from one interface to the other in the absorption pipette, but errors as great as 0·1% are not important in factory control of gas packing. During trials at a milk drying plant, air was frequently analysed for check purposes and in all cases figures between 20·95 and 21·04% were obtained.

With careful manipulation the apparatus may also be used to check the oxygen content of nitrogen used for gas packing. Two copper side tubes are sweated into a small sealed tin and the cylinder gas passed in through one tube and out through the other for a minute or two to sweep out air. With the gas still flowing a sample may then be withdrawn from the tin in the usual way. Before taking a duplicate sample the first hole made by the needle should be soldered.

When carrying out a series of determinations it is not necessary to check for complete oxygen absorption each time; it is sufficient to check at the first analysis of a series and then at every tenth one.

Summary

An apparatus has been developed for the routine estimation of oxygen in the headspace gas of tins of dried milk. The sampling device is an integral part of the apparatus, which is constructed mainly of metal and is simple to operate. As many as twenty samples may be taken and analysed in an hour.

Our thanks are due to the Drawing Office and Workshop staffs of the Division of Industrial Chemistry, Council for Scientific and Industrial Research, who designed and manufactured the equipment, and to the Maffra Co-operative Milk Products Co. Ltd., who permitted the apparatus to be tested in their laboratories under factory conditions.

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637.143

358. THE EFFECT OF A HIGH PRE-HEATING TEMPERATURE WITH AND WITHOUT ETHYL GALLATE ON THE STORAGE LIFE OF WHOLE-MILK POWDER SPRAY-DRIED ON A GRAY-JENSEN DRIER*

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(With 5 Figures)

Introduction

In a large-scale experiment carried out a few years ago by Mattick *et al.* (1) spray-dried whole milk manufactured by the Kestner process from milk which had been pre-heated to 190° F. was found to keep in good condition for 2–3 times as long as powder made on the same plant from milk which had been pre-heated to a temperature of only 165° F. Later, Findlay *et al.* (2) studied the effect of various pre-heating temperatures on the keeping quality of spray-dried whole milk made by the Krause process. They found that the storage life of the product was greatly extended by increasing the pre-heating temperature to 190° F., but that no additional advantage was gained by raising it further to 200° F. In both these series of experiments the reconstituted milk made from the high-temperature powders had a slight 'cooked' or 'processed' flavour which was not noticeable in the reconstituted milks made from the low-temperature powders. The 'cooked' or 'processed' flavour in the reconstituted milks was not unpleasant, but it had the disadvantage that it reduced their resemblance to fresh liquid milk.

In earlier work on the effect of antioxidants on the keeping quality of milk powder Findlay *et al.* (3) found that the presence of 0·07% ethyl gallate in dried whole milk greatly retarded the development of tallowiness and thus extended the storage life of the powder 2½–3 times when stored at 37° C. From this observation the question arose as to whether a powder of good keeping quality, but without a 'cooked' flavour, could be obtained by using ethyl gallate together with a low pre-heating temperature. In order to investigate this problem it was decided that the two methods of improving the keeping quality should be compared under adequately controlled conditions for the same batch of liquid milk dried on the same plant. The present experiments were therefore planned in such a way that the effect of using a high pre-heating temperature could be compared with that of using ethyl gallate in conjunction with a low pre-heating temperature. At the same time the opportunity was taken of determining whether the addition of ethyl gallate to a high temperature pre-heated milk would extend the storage life of the powder still further than the use of a high pre-heating temperature alone.

A Gray-Jensen plant was chosen for the work, since the effect of high and low pre-heating temperatures had not so far been compared under strictly controlled conditions for this type of plant.

* Attention is called in connexion with this work to the Public Health (Preservatives, etc., in Food) Regulations made by the Ministry of Health.

Drying the milk

Through the helpful co-operation of the Scottish Milk Powder Co. Ltd., a Gray-Jensen plant was made available for the experiments. Owing to the very small amount of milk which had to be dried, the pre-condensing stage was omitted, but this is unlikely to render the general conclusions inapplicable to the ordinary Gray-Jensen process in which the milk is pre-condensed in a liquid collector.

A total of 500 gal. of raw milk was run into a tank and circulated through a heater at 160° F. and returned to the tank until all the milk had reached a temperature of 160° F. Heating was then stopped, and 150 gal. of the milk were transferred to a glass-lined tank from which they could be pumped through the 'atomizer' into the drying chamber. 50 gal. of the milk were first dried and the powder discarded. The next 50 gal. were dried and the product collected to serve as the low-temperature control sample. To the remaining 50 gal. of milk 25 g. of ethyl gallate dissolved in 500 ml. water were added and the mixture well stirred. This third batch of 50 gal. was then dried, only the product emerging from the drier towards the middle of the brief drying period being collected as the low-temperature ethyl gallate sample.

The remaining 350 gal. of milk were circulated through the heater at 190° F. until the whole volume had been maintained at that temperature for 5 min., when the temperature was allowed to fall. Five minutes is a much longer period of heating than was used in the previous work (1, 2), but it was adopted in the present experiment in order to make sure, with the particular type of heater available, that all the milk had attained 190° F. 150 gal. of the heated milk were then transferred to a clean glass-lined tank and dried as before in three batches of 50 gal. each.

The four samples of dried milk were collected at the bottom of the drying chamber, thoroughly mixed and transferred to 21 lb. cans. Half of each sample was sent to the Low Temperature Research Station, Cambridge, and half to the Hannah Dairy Research Institute. The storage tests carried out at the two Institutes were similar to those already described in earlier work (1, 2).

Properties of the fresh powders

Ethyl gallate content. The amount of ethyl gallate in the powders was estimated as previously described (3). In the low-temperature ethyl gallate powder the ethyl gallate content was 0·08%, and in the corresponding high-temperature powder 0·06%. It was estimated again once or twice during the storage period, but even after 8 months at 47° C. or 1½ years at 37° C. it remained unaltered, thus confirming the observation recorded in the earlier experiments (3) that the amount of gallate present does not appear to diminish on storage. No gallate was present in either the low- or the high-temperature control powder.

Moisture content. The moisture contents of the powders by the air-oven method are recorded in Table 1, values determined by vacuum oven being, on the average, 0·2% higher. Since none of the values exceeded 1·3%, it was to be expected that the predominating type of spoilage would be fat deterioration, rather than the protein-lactose type of degradation which is very marked in powders of high moisture content (4).

Copper content. The copper content of the powders was estimated by the method of Hetrick & Tracy (5). The results recorded in Table 1 show that in the low-temperature

control there was a minimal copper content of 1·4 p.p.m. In the low-temperature ethyl gallate powder and the high-temperature control powder the values were but little higher and almost equal. In the fourth powder, however, where both a high pre-heating temperature had been used and ethyl gallate had been added, the value rose to 3·8 p.p.m.

The fact that the lowest copper content occurred in the low-temperature control sample means that the beneficial effects attributed in the present work to the addition of ethyl gallate or to raising the pre-heating temperature or to both would probably have been slightly greater if the copper content of all four samples had been uniformly low.

Readily extractable or 'free' fat. In some of the experiments of this series (2) the keeping quality of the milk powders examined has tended to be better in lacquered than in plain tinplate cans. It was thought that the degree to which the fat was readily extractable by organic solvents might have some bearing on this finding, and in the present work values for the free fat (Table 1) have been determined by extraction with light petroleum both at room temperature for 5 min. and at 40–60° C. for 5 hr.

Table 1. *The properties of fresh powders*

Pre-heating temperature (° F.)	Ethyl gallate content (%)	Moisture (%) (air oven)	Copper content (p.p.m.)	Fat extracted by light petroleum	
				Cold* (%)	Hot† (%)
160	None	1·1	1·4	6·0	13·9
160	0·08	1·2	2·3	6·3	13·6
190	None	0·8	2·1	5·1	11·3
190	0·06	1·1	3·8	4·8	10·7

* 5 min. at room temperature. † 5 hr. at 40–60° C.

Sulphydryl test. In the previous work (1, 2) the extended storage life of the high-temperature powders was associated with a marked positive sulphydryl test (6) as indicated by the development of a pink colour on treatment under suitable conditions with sodium nitroprusside. The presence of volatile sulphur compounds in the high-temperature powders in one series of experiments (2) was also shown by a semi-quantitative modification of the methylene-blue method (7). In the present investigation the liquid milk pre-heated to 190° F. gave a positive sulphydryl test before it was dried, but with the dried product no pink colour could be obtained. On a previous occasion when the nitroprusside test was applied to Gray-Jensen powder by the present authors similar observations were made. It may be that under the influence of the high-pressure 'atomizer' the particular volatile sulphur compounds which are responsible for the pink coloration are driven off in the current of hot air during the drying process. However true this suggestion may be, the fact remains that notwithstanding the absence of the characteristic pink colour in the qualitative sulphydryl test, the high-temperature powders still showed the greatly improved keeping quality which can now be regarded as typical of powders for which high pre-heating temperatures have been used.

Flavour. The flavour of all the powders when reconstituted immediately after manufacture was satisfactory. As usual the high pre-heating temperature had imparted a slight but not unpleasant cooked flavour to the product. For this reason there was a tendency for the low-temperature powders to be preferred at the beginning of the storage period, but subsequently they deteriorated much more rapidly than the high-temperature powders. The ethyl gallate was not found to cause any degree of unpalatability in the powders.

Biological value of the protein. Estimation of the biological value of the protein of the low- and high-temperature control powders by Henry & Kon (8) disclosed no significant difference between them.

Storage tests at the Hannah Institute

The powders were packed in plain tinplate containers and also in containers of tinplate coated with meat lacquer (a phenol-formaldehyde thermo-setting resin). They consisted of 6 oz. Danish cream cans of the 'sanitary, open-top' type. Each can contained 75 g. powder, and since the volume of this type of can is 186 ml. the packing density was 0·403 g./ml. can space. Assuming an apparent density of 1·33 for the milk solids (9) the free oxygen content of the packed cans was 48·3 mg. O_2/100 g. powder. After the seams of the cans had been treated externally with bitumen to ensure gas-tightness, the cans were stored in water-jacketed incubators at 47 and 37° C., and also at room temperature. A number of cans both air-packed and nitrogen-packed were kept in a refrigerator to act as controls.

Peroxide values were determined at intervals throughout the storage period by the method of Chapman & McFarlane (10) with two slight modifications. First, the moisture content of the powder was raised to 10 or 12% (in a humid atmosphere of nitrogen) to facilitate extraction of the fat, and secondly, in extracting the fat, the acetone was brought just to the boil in the presence of the powder, the mixture shaken well, cooled and filtered. It has been found that with these modifications it is unnecessary to reflux with acetone for 20 min. as described in the original method. Values given by the Chapman & McFarlane method are probably too high (11), but, used in a relative sense, they supply useful information as to the degree of oxidation of the fat (2).

For the tasting tests on the reconstituted milks a procedure identical with that outlined in previous papers (1, 2, 3) was followed, marks being awarded by the panel members according to the scheme: 0 = very good and very like ordinary fresh milk, 1 = fairly good and quite palatable, 2 = slightly but definitely unpalatable due to the presence of slight off-flavours, 3 = unpalatable due to the presence of pronounced off-flavours, 4 = very tallowy and most unpalatable. A control powder which had been stored at 0° C. was included in each test.

The average of the off-flavour marks awarded by the five tasters for each sample at each tasting was plotted against the storage time to give the curves shown in Fig. 1. When the curves so obtained pass an off-flavour score of 1·0, the trained panel is beginning to detect the development of slight off-flavours. When score 2·0 is reached the off-flavours are believed to be sufficiently obvious to be noticed and objected to by the ordinary consumer. The times required for off-flavour marks of 1·0 and 2·0 to be passed have been recorded in Table 2.

The amount of oxygen absorbed by the various powders has been plotted in a similar way (Fig. 2). From the graphs so obtained the times taken for 10 and 20 mg. oxygen to be absorbed per 100 g. powder have been taken for comparing the rates at which the powders deteriorated. These times are recorded in Table 3. The temperature coefficients for deterioration in flavour and for absorption of oxygen are shown in Table 4 and peroxide values in Fig. 5.

Storage tests at Cambridge

The four powders were packed in 240 ml. cans at the rate of 96·8 g. per can, corresponding to a packing density of 0·403 g./ml. and a free oxygen content of 48·3 mg./100 g. powder. Both plain tinplate and a natural oleo-resinous ('fruit') lacquered tinplate were used. The end seams of the cans were coated with Wood's metal and bitumen and the side seams with bitumen to ensure gas-tightness.

The cans were stored in constant-temperature rooms at 37 and 15° C., gas-packed samples being kept at −20° C. for use as controls. The stored powders, after reconstitution, were graded by tasting panel according to the scale 6 = very good and equal to fresh milk, 5 = good, 4 = fairly good, 3 = fair, 2 = rather poor, 1 = poor, 0 = very poor, a system which has been described more fully elsewhere (1, 2).

The tasting results are shown in Fig. 3, and the oxygen absorption results in Fig. 4.

The times required for the powders to deteriorate to flavour scores of 4 and 2 are given in Table 2. A flavour score of 4 is considered to denote the end of the storage life of the powder as a product of reasonably good quality, while at 2 the reconstituted milk is becoming definitely unpleasant. The times are recorded in weeks and also as a multiple of the storage life of the least stable sample of the series. Similar data for absorption of oxygen are given in Table 3, and temperature coefficients for deterioration in flavour and absorption of oxygen are recorded in Table 4.

Results

Flavour. The results of the tasting tests (Figs. 1 and 3 and Table 2) show that raising the pre-heating temperature of the liquid milk from 160 to 190° F. *or* the addition of ethyl gallate to the milk before drying extended the storage life of the control powder by a factor of the order of 1½–2½ at 47° C., 2–3 at 37° C. and 3–4 at 15° C. or room temperature. These observations are in good general agreement with the previously recorded effects of high temperature pre-heating (1, 2) and of the incorporation of ethyl gallate (1, 3) on the stability of spray-dried full-cream milk powder of low moisture content.

Increasing the pre-heating temperature *and* incorporating ethyl gallate in the milk extended storage life by factors of the order of 3 at 47° C., 4 at 37° C. and 8 at 15° C.

Absorption of oxygen. Oxygen absorption data (Figs. 2 and 4 and Table 3) confirmed the beneficial effects of high-temperature pre-heating and of the incorporation of ethyl gallate, as well as the further increase in stability obtained when both means were employed together. Protection factors too were progressively higher as the temperature of storage decreased from 47° C., through 37 to 15° C. or room temperature, as in the flavour tests. Improvements in keeping properties when oxygen absorption was used as criterion were, however, considerably smaller than when the samples were graded by tasting panel.

It was again noted that while there was a general parallelism between oxygen absorption and development of off-flavour, the ratio of the quantity of oxygen absorbed to the degree of off-flavour produced was not constant. High-temperature pre-heating, incorporation of ethyl gallate and high storage temperature all tended to increase the amount of oxygen required to produce a given degree of off-flavour, presumably by retarding oxidation of the fat and/or by increasing the proportion of the absorbed oxygen reacting with non-fatty constituents of the powder.

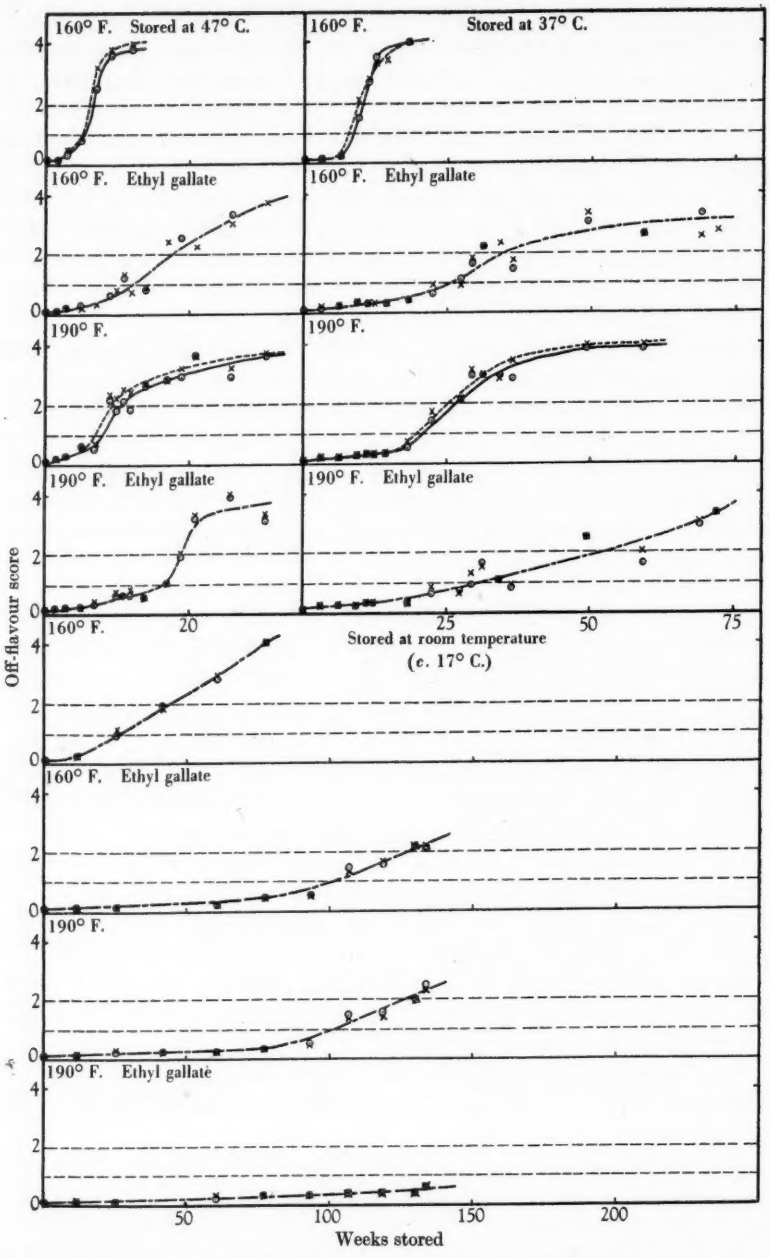

Fig. 1. Changes in the palatability of full-cream milk powders prepared from milk pre-heated at 160 and 190° F., with and without ethyl gallate, during storage at 47, 37° C. and room temperature (*c.* 17° C.). (Hannah Institute results.)

⊙ = lacquered tinplate can. × = plain tinplate can.

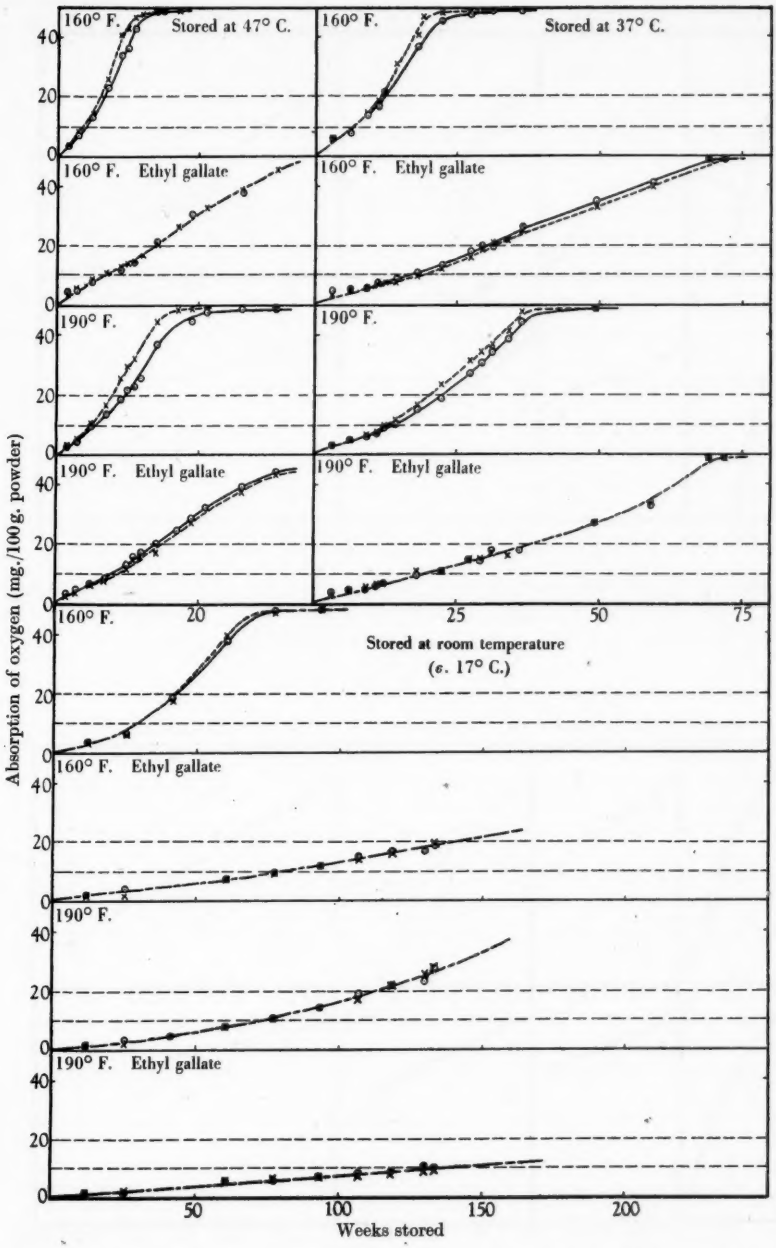

Fig. 2. Absorption of oxygen by full-cream milk powders prepared from milk pre-heated at 160 and 190° F., with and without ethyl gallate, during storage at 47, 37° C. and room temperature (*c.* 17° C.). (Hannah Institute results.)

⊙ = lacquered tinplate can. × = plain tinplate can.

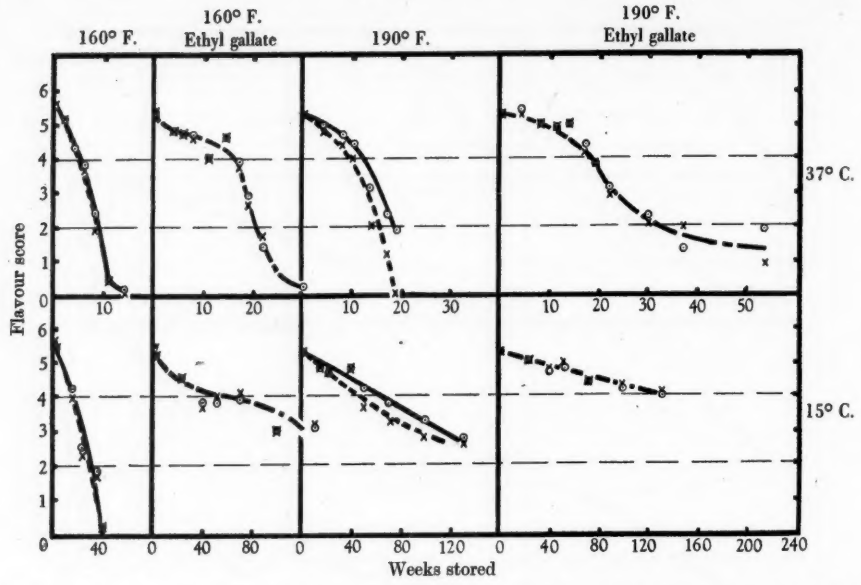

Fig. 3. Changes in the palatability of full-cream milk powders prepared from milk pre-heated at 160 and 190° F., with and without ethyl gallate, during storage at 37 and 15° C. (Cambridge results.)

⊙ = lacquered tinplate can. × = plain tinplate can.

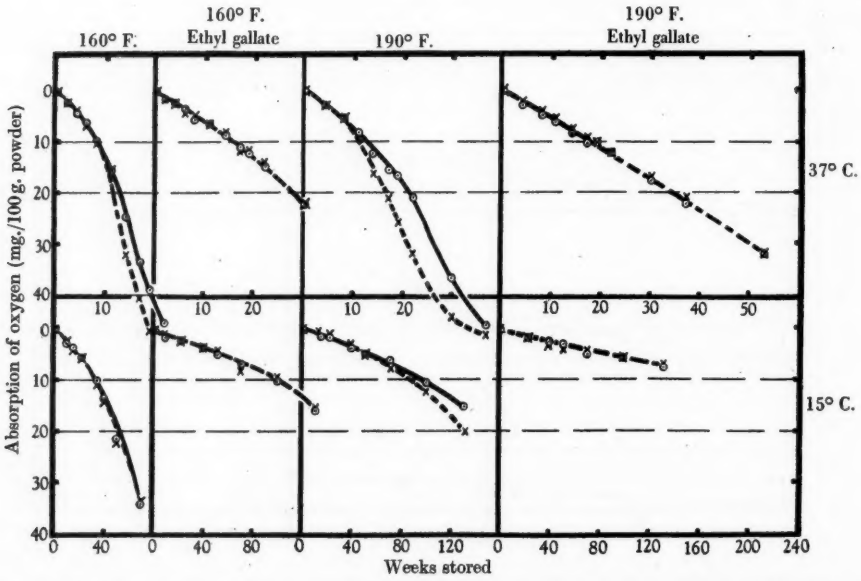

Fig. 4. Absorption of oxygen by full-cream milk powders prepared from milk pre-heated at 160 and 190° F., with and without ethyl gallate, during storage at 37 and 15° C. (Cambridge results.)

⊙ = lacquered tinplate can. × = plain tinplate can.

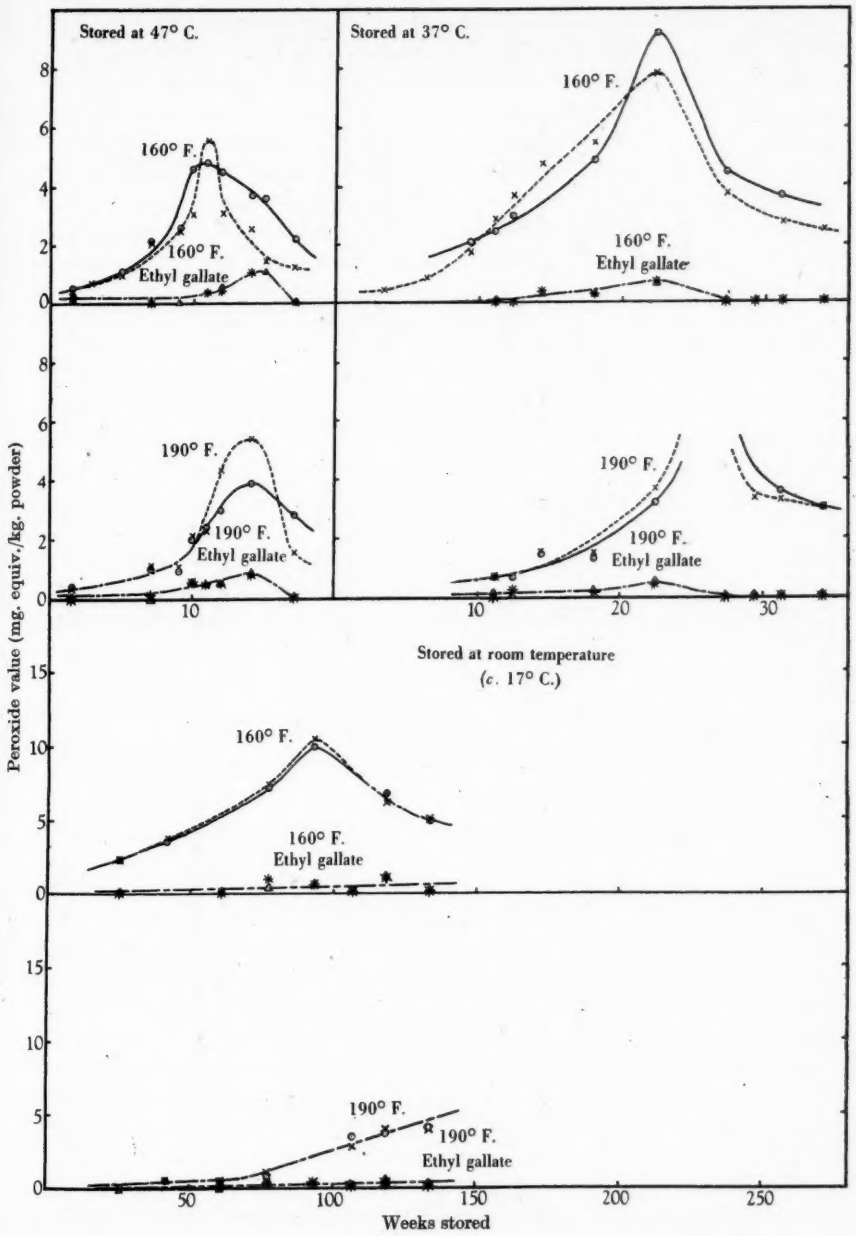

Fig. 5. Peroxide values of full-cream milk powders prepared from milk pre-heated at 160 and 190° F., with and without ethyl gallate, during storage at 47, 37° C. and room temperature (c. 17° C.). (Hannah Institute results.)

⊙, △ = lacquered tinplate can. ×, * = plain tinplate can.

Table 4. *Temperature coefficients* for deterioration in flavour and absorption of oxygen*

Pre-heating temp. (° F.)	Ethyl gallate (%)	Hannah Institute results								Cambridge results				Average
		Measured at off-flavour score 1·0				Measured at off-flavour score 2·0				Measured at flavour score 4·0		Measured at flavour score 2·0		
		Lacquered cans		Plain cans		Lacquered cans		Plain cans		Lacquered cans	Plain cans	Lacquered cans	Plain cans	
		47–37° C.	37–17° C.†	47–37° C.	37–17° C.	47–37° C.	37–17° C.	47–37° C.	37–17° C.	37–15° C.	37–15° C.	37–15° C.	37–15° C.	
160	None	1·6	1·8	1·5	1·8	1·5	2·1	1·6	2·2	1·7	1·7	1·8	1·8	1·8
160	0·08	2·0	2·0	2·0	2·0	1·8	2·0	1·7	2·1	1·8	1·8	—	—	1·9
190	None	2·5	2·2	2·5	2·3	2·5	2·2	2·7	2·2	2·1	2·0	—	—	2·3
190	0·06	1·9	—	1·8	—	2·1	—	2·0	—	2·4	2·5	—	—	2·1
		Measured at 10 mg. O_2/100 g. powder				Measured at 20 mg. O_2/100 g. powder				Measured at 10 mg. O_2/100 g. powder		Measured at 20 mg. O_2/100 g. powder		
160	None	1·8	2·0	1·8	2·2	1·9	1·8	2·0	1·8	1·9	1·9	1·9	2·0	1·9
160	0·08	2·1	2·2	2·8	2·1	2·2	1·6	2·3	1·6	2·3	2·3	—	—	2·2
190	None	2·6	2·3	2·6	2·5	2·3	1·8	2·6	1·9	2·5	2·5	—	2·5	2·4
190	0·06	2·7	2·6	2·4	2·5	2·7	—	2·6	—	2·8	2·8	—	—	2·6

* Defined as the ratio of the times required to produce the specified change at two temperatures 10° C. apart.

† It is assumed that the average room temperature approximated to 17° C.

These findings are in general agreement with observations in the previous work on powders made by the Krause process (2), where possible causes are discussed.

Effect of the container on keeping properties. The figures and tables already cited show that in the two control powders, which contained no ethyl gallate, the storage life as determined independently at the two laboratories appeared to be about 10% higher in lacquered than in plain tinplate. There was therefore an advantage in favour of lacquered tinplate, but it was small compared with that recorded in earlier work with powder made on a Krause plant (2), and with that observed in previous, unpublished experiments with Gray-Jensen powder. It is not at present known whether the large variations observed in the magnitude of the 'container effect' is due to variation in the surfaces used, to variation in the physical or chemical structure of the powders, or to some factor such as degree of agitation which might vary in different experiments.

The powders containing ethyl gallate showed practically no container effect, the pro-oxidant action of the tinplate surface presumably being neutralized by the presence of the fat-soluble antioxidant. The effect of tinplate and of lacquered surfaces on the oxidative deterioration of butterfat has been discussed recently by one of us (12).

Peroxide values. The peroxide values given in Fig. 5 show that the fat in the control powder made from milk pre-heated to 190° F. was more resistant to oxidation than the fat of the corresponding powder for which the pre-heating temperature was 160° F. They also show that the addition of ethyl gallate increased still further the resistance of the fat to oxidation. The peroxide values for the powders in plain tinplate did not differ significantly from those for the corresponding powders in lacquered cans, but where any measurable difference was observed oxidation in the lacquered cans was usually slightly less than in the plain cans. In all these respects, therefore, the peroxide values confirm the results of the tasting and oxygen absorption tests.

Temperature coefficients. The relationship between the rates of deterioration at the various storage temperatures is shown in Table 4. As in the earlier work with Krause powder (2) there is a distinct tendency for the temperature coefficients to be lower for the low-temperature pre-heated samples than for the high-temperature pre-heated samples.

Discussion and conclusions

One of the most difficult problems in the present work has been that of assessment of the relative merits of high-temperature pre-heating without ethyl gallate and of low-temperature pre-heating with gallate, and no very definite conclusion has been reached. Both the oxygen absorption and the taste panel methods agreed in favouring the gallate-treated powder when the temperature of storage was high, but at 15° C. or at room temperature the differences were small and, by taste, erratic.

Tasting by a small trained panel is only of limited value when comparison has to be made between materials such as the two powders in question, which were initially somewhat different in flavour and which developed notably different off-flavours on storage. The low-temperature pre-heated, gallate-treated sample speedily developed a rather marked flavour and (more especially) odour somewhat similar to the 'incipient tallowy' taint which has been noticed in fresh spray-dried commercial powder made from low-temperature pre-heated milk on a plant which slightly contaminates the product with copper. On the other hand, the flavour of this milk contained certain elements of 'cool-

ness' and 'freshness' not noticeable in the partly deteriorated high-temperature powder, and it showed considerable resistance to the development of strong tallowy off-flavours. Opinions as to degree of undesirability of the incipient tallowy flavour varied markedly with the individual, and the Cambridge panel experienced some difficulty in making up its mind about the acceptability of some of the samples (cf. Fig. 3, 15° C. results). No attempt at a consumer test was made, but it seemed possible that the average person might tend to object more strongly to this rather unusual, although to many people not unpleasant flavour than to the rather 'stale', and 'milk pudding-like' tastes developed by high-temperature pre-heated milks in the earlier stages of deterioration. It is tentatively concluded, therefore, although perhaps slightly against the balance of the numerical evidence produced, that for normal use low-temperature pre-heating plus ethyl gallate is *not* to be preferred to high-temperature pre-heating without gallate.

No difficulty was experienced in grading the high-temperature pre-heated, gallate-treated sample which, as already stated, possessed exceptional keeping properties. A possibility would seem to exist of using high-temperature pre-heating plus gallate as an alternative to high-temperature pre-heating plus gas packing for moderate periods of storage.

Summary

1. Storage tests have been made independently in two laboratories on four samples of spray-dried whole-milk powder prepared on a Gray-Jensen plant from one batch of milk using pre-heating temperatures of 160 and 190° F. with and without the addition of 0·06–0·08% of ethyl gallate as antioxidant. The powders were packed in lacquered and in plain tinplate containers and stored at 47, 37 and 15° C., and at room temperature. Deterioration was followed by tasting tests, by determination of the amount of oxygen absorbed by the powders and by estimation of peroxide in the fat.

2. Raising the pre-heating temperature from 160 to 190° F. *or* the addition of ethyl gallate to the milk improved the keeping quality of the resulting powder, as measured by taste, by a factor of the order of $1\frac{1}{2}$–$2\frac{1}{2}$ at 47° C., of 2–3 at 37° C. and of 3–4 at 15° C. and room temperature.

3. Increasing the pre-heating temperature *and* incorporating ethyl gallate in the milk extended the storage life of the powder by a factor of the order of 3 at 47° C., 4 at 37° C. and 8 at 15° C. and room temperature.

4. For an equivalent loss of palatability powder from high-temperature pre-heated milk absorbed appreciably more oxygen than the corresponding powder from low-temperature pre-heated milk, and the gallate-treated samples slightly more than the corresponding control powders.

5. The temperature coefficients for deterioration were slightly higher for the high-temperature powders than for the corresponding low-temperature powders.

6. With the two control powders storage in lacquered tinplate resulted in a small increase, of the order of 10%, in keeping properties as compared with storage in plain tinplate. With the ethyl gallate-treated powders no advantage resulted from the use of lacquered tinplate.

The authors wish to express their indebtedness to the Managing Director of the Scottish Milk Powder Co. Ltd., and to the Company's Manager at Kirkcudbright for their helpful co-operation in preparing the powders. The work described above forms part

of a joint programme of the Hannah Dairy Research Institute, the Agricultural Research Council and the Food Investigation Board, Department of Scientific and Industrial Research.

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359. A NOTE ON THE EFFECT OF THE TEMPERATURE OF PRE-HEATING OF SPRAY-DRIED WHOLE MILK ON THE BIOLOGICAL VALUE OF ITS PROTEINS

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Previous work from this laboratory has indicated that the biological value of the proteins of milk is not affected by pasteurization (1), by evaporation and condensation (2) or by spray or roller-drying (3). Sterilization (4), on the other hand, caused a statistically significant decrease of 6% in the biological value; with this process heating at a high temperature is more prolonged than in the other forms of heat treatment.

It was of interest to find out whether the difference of 30° F. in the pre-heating temperature of the spray-dried whole milks described by White, Smith & Lea (5) in the preceding paper would have any effect on the biological value of the proteins.

Experimental

The biological value and true digestibility of the proteins of the control milks pre-heated to 160 and 190° F. (5) were determined on rats by the method of Mitchell (6, 7). Two litters of two, and two litters of four, female rats 38–40 days old and weighing 115–132 g. were used. The experimental technique was that fully described by Henry, Kon & Watson (1). The milks were fed at an 8% level of protein intake (N × 6·38). Sufficient margarine fat was added to the experimental diets to bring their total fat content to approximately 10%. Their composition and analysis is given in Table 1.

Table 1. *Composition and analysis of the experimental diets*

Component	Diet 261	Diet 262
Spray-dried whole milk, pre-heated to 160° F.	28·0	—
Spray-dried whole milk, pre-heated to 190° F.	—	27·9
Salts (de Loureiro (8))	4·0	4·0
Margarine fat	3·0	3·0
Sugar, granulated, ground	12·0	12·0
Potato starch	10·0	10·0
Rice starch	43·0	43·1
% nitrogen	1·280	1·267
% moisture	8·26	8·02

The results of the experiment are shown in Table 2. It will be seen that the milk with the high pre-heating temperature gave slightly lower values both for the biological value and true digestibility, but the small differences observed are without statistical significance.

This result was not unexpected as in our previous experience sterilization (4) was the only form of heat treatment which had a deleterious effect on the biological value. In the course of this process the milk is subjected to a temperature of about 230° F. for prolonged periods. No ill effects were noted after evaporation (2, 3) when the milk was maintained at a temperature of 235–240° F. for 15–20 min. It would appear, therefore,

that protein injury is only likely to occur when the heating at high temperatures is prolonged. The 'high-temperature' milk used in the present test was held at a temperature of 190° F. for 5 min. prior to drying; this was rather longer than is usual in commercial practice (5).

Table 2. *Mean figures, with their standard errors, for the biological values and true digestibilities of the proteins of the milks pre-heated to high and normal temperatures. Statistical treatment* of the results*

	Biological value		True digestibility	
Pre-heating temperature of milk...	160° F.	190° F.	160° F.	190° F.
1st experimental period	93·9±1·37	92·0±1·80	94·2±0·50	91·9±0·61
2nd experimental period	84·1±2·16	83·4±2·19	89·3±0·86	88·6±1·09
Mean	89·0±1·93	87·7±1·87	91·8±0·88	90·3±0·77
Difference: 160–190° F.	1·3		1·6	
S.E.M.	±3·3		±1·0	
P†	1 : 1 N.S.		1 : 7 N.S.	

* The paired t-test of 'Student' (9, 10) was used. Comparisons were made between values obtained for each milk with the same rat.

† P = probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population. N.S. = not significant.

It is safe to conclude that the biological value and true digestibility of the proteins of milk pre-heated to 190° F. are not inferior to those of milk pre-heated to the lower temperature of 160° F.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION A. PHYSIOLOGY OF DAIRY CATTLE

II. NUTRITION

General considerations and feeding standards

Crampton (1) has criticized the orthodox methods of describing the value of feeding stuffs and has proposed an alternative scheme. He states, however, that any chemical analysis of feeding stuffs is at best only a crude indication of their value to the animal. Similar criticisms of orthodox methods come from Lancaster (2) and from Louw (3) who carried out digestibility trials with sheep. Lancaster, using nine succulent feeds and five different roughages, found high negative correlations between lignin percentage and the digestibility of organic matter and of cellulose. Crampton & Whiting (4) recommend that in describing the proximate constituents of feeds, a determination of their lignin content should be included as an index of relatively indigestible residues. Even lignin (5), however, undergoes some digestion by the ruminant, so that the estimation of digestibilities by means of lignin ratios is open to objection. The use of lignin is open to the additional objection that there are difficulties in its accurate determination (6).

Crampton & Whiting determined ether extract on dried material. Ether extraction of plant as well as of animal materials is better done on moist than dried samples. Constituent silica has again been suggested as a marker in digestibility trials with farm animals (7), but this is open to objection because of the inevitable contamination of practically all plant foodstuffs with soil. Moreover, particles of such soil may well be retained in the rumen because of their relatively large density.

Lucas (8) has described a method of equalized feeding for studies with dairy cows. Jarl (9) found that overfeeding above maintenance in the dry period was detrimental to the subsequent lactation, though with first-calving heifers there was an increased yield. In the parallel field of sheep husbandry, Briggs, Darlow, Hawkins, Willham & Hauser (10) claim that it is not economic to feed extra rations to ewes just before lambing. On the other hand, Underwood & Shier (11) found that it was profitable to feed ½ lb. of wheat in the last 4 weeks before lambing, since it reduced the incidence of pregnancy toxaemia. In a later study Underwood, Shier & Cariss (12) found that feeding of ½ lb. wheat and ½ lb. linseed nuts in the last 2 months of pregnancy had a favourable effect in reducing losses of ewes from pregnancy toxaemia and of lambs at or near lambing time. There was a highly significant increase in the birth weight and growth rate of lambs. Such results are borne out by those of Wallace (13), who showed that extra feeding of ewes during the last 2 months of pregnancy inereased both the weight of the lambs at birth and the milk yield of their dams. Wallace also observed that extra feeding during the last 2 months was much more effective than earlier in pregnancy, and that such feeding increased foetal weight more markedly when practised in late pregnancy. The increased milk yields were largely due to increased growth of the secretory tissues of the mammae.

Blaxter (14) found that bulky foods are often partly refused and so reduce milk production. He also found (15) that the yields of cows to which extra bulky foods or extra concentrates had been fed for 6 weeks prior to calving were greater than those of comparable control animals. The cows receiving concentrates gave more milk than those which received extra bulky foods. Attempts by Blaxter (16) to increase yields by overfeeding with bulky foods were unsuccessful because the cows refused to eat the extra food. Increase of concentrates in mid-lactation produced a negligible response. Thus in times of scarcity, concentrates should be used just before calving and not in mid-lactation. The basal standard of rationing greatly affects results obtained

from this type of experiment, and the biological optimum is not generally the economic optimum (17, 18). In this connexion it should be noted that the law of diminishing returns (17, 18, 19) applies to the feeding of animals for milk production, and that the results of feeding experiments will depend on whether the animals are fed to capacity (as Blaxter's evidently were) or are more strictly rationed either by man or, in the case of most sheep, by the nature of their environment. Blaxter & French (20) tried the effect of feeding bulky foods at varying intervals during the day with a view to increasing the yield of milk from them. The experiment was unsuccessful. Bonnier (21) claims that rationing, as usually practised, prevents cows from yielding milk to their full inherited capacity. The relative nutritive values of roughage, concentrates and pasture are discussed by Huffman & Duncan (22).

Gaines (23) has fitted the following equations to published data on the milk production of cows, goats and rats respectively:

$$d = 0{\cdot}008W + 0{\cdot}3F, \quad d = 0{\cdot}016W + 0{\cdot}3F, \quad d = 0{\cdot}064W + 0{\cdot}3F,$$

where d is the intake of digestible nutrients, W is the live weight and F is the yield of milk calculated to 4% fat content, the unit of weight being the same in all three measurements. Gaines (24) in another paper joins issue with Kleiber by maintaining that the energy requirement for maintenance for a cow in milk is proportional to its weight at the beginning of lactation and not to $W^{\frac{3}{4}}$ as held by Kleiber. Gaines states, however, that he is chiefly interested in comparing the yield of one animal with that of any other animal of the same species. For further discussion of the pros and cons of this argument reference should be made to Brody (18), who does not fail to emphasize the theoretical difficulties involved in estimation of energy needs. In the same book a valuable up-to-date discussion of the theory of feeding standards is given.

Kriss (25) has presented a report of an American committee on the evaluation of feeds on the basis of net available nutrients. Questions still needing investigation receive attention in this report. By means of energy balances, corrected for storage, Forbes & Thacker (26) have shown that, when a factor of 3·3 instead of 7·8 was employed for the value of the ether extract of silage, the metabolizable energy of a basal ration and of various silages could be predicted by the use of the factors of Axelsson (27). Forbes & Swift (28) also found that timothy hay was richer in digestible nutrients and metabolizable energy than alfalfa hay, but not so rich in protein. The digestible nutrients and the metabolizable energy of maize plus timothy hay were greater than those of maize plus alfalfa hay, while, judged by these same two criteria, alfalfa silage was better than alfalfa hay. Lime caused no increase in either metabolizable energy or digestible nutrients when added to phosphoric acid silage. Woodman & Evans (29) report that the increase of digestibility resulting from milling wheaten offals does not repay the added cost of power and labour it involves. They give a graph which can be used to predict the starch equivalent of such offals from their fibre content.

Johnstone-Wallace & Kennedy (30) have made observations of the grazing habits of cattle. They found that on fresh young pasture the animals spent roughly a third of their time grazing, a third ruminating and a third lying down. They traversed 2½ miles per day in grazing, and when grass was 4–5 in. high ingested per day 150 lb. of green herbage (= 32 lb. dry matter). Such a large intake of herbage, say the authors, is only possible on fresh young pasture which is eaten down in a day or two.

As regards calf feeding Blizzard & Taylor (31) found oats to be superior to maize when fed with molasses, cottonseed cake and hay to calves as judged by weight increase. The effect of the plane of nutrition on the comparative growth rates of nine pairs of monozygotic twin heifer calves from birth to maturity has been studied by Bonnier & Hanson (32). One sister of each pair was overfed to the same extent as her twin was underfed. The higher plane of nutrition produced a greater rate of growth, but if allowance was made for maintenance those on the lower plane used less food per unit increase of weight. Genetic limitations of final weight were shown by comparing the fastest growing pair with the slowest growing pair.

The utilization of non-protein nitrogen

Urea, if fed in a suitable mixture and in the presence of readily available carbohydrate, such as starch, can replace part of the protein in the rations of ruminants, for both growth and milk production (33). This is true even when the protein replaced is of high nutritive value. Non-ruminants cannot use urea in this way (34, 35, 36, 37, 38).

It appears from recent work (39, 40) that non-protein nitrogen is utilized by ruminants because the rumen abounds with iodophilic bacteria. In the presence of added urea and added available carbohydrate the multiplication of these iodophilic bacteria leads to the synthesis in their bodies of considerable amounts of 'bacterial starch' and, of course, of protein. Ammonia derived preferably from intraruminal hydrolysis of urea can serve as a source of nitrogen for these bacteria. The source of carbohydrate seems to be of a more specific nature. Thus *in vitro* starch and various sugars, such as maltose and galactose, may provide for the energy requirements of the iodophile bacteria of the rumen (39). When, however, instead of receiving sugar or starch the bacteria are supplied with glycerol, multiplication does not occur and protein synthesis is replaced by proteolysis. It may well be that, while hexoses or more complex carbohydrates are available either for synthesis of bacterial protoplasm or for breakdown into fatty acids (41, 42, 43, 44, 45, 46), glycerol is only available for the latter process.

Pearson & Smith (39) are of the opinion that analyses of materials abstracted through rumen fistulas may often be subject to large and relatively uncontrollable errors of sampling, as, indeed, McElroy & Goss (46) admit. Pearson & Smith (39) have therefore preferred to incubate rumen contents *in vitro* using the microscopic appearance of the bacterial flora and the *p*H of such incubated material as criteria of normality. They found that under certain easily reproducible conditions the chemical and bacteriological nature of such material is still comparable with normal rumen contents after 3–4 hr. of incubation *in vitro*. Using this method of incubation they have shown that urea added to rumen contents is rapidly hydrolysed to ammonia. They have also shown (39) that hydrolysis and synthesis of protein can both occur in the rumen, synthesis of protein being favoured by the addition of urea and a readily available carbohydrate.

Smith & Baker (40) investigated the correlation between bacterial multiplication and protein and polysaccharide synthesis in the rumen. Counts of Protozoa, and of each of several morphologically distinct types of bacteria, were recorded both before and after incubation. The volume of zoogloea was also determined. All these, except the Protozoa, increased on incubation, the contribution made by *Amylococcus* chains being greatest. When material consisting mainly of *Amylococcus* chains was isolated by centrifugation of material freshly removed from the rumen, it was found to be capable of a twelvefold increase by reproduction when incubated *in vitro*. Chemical analysis showed that protein synthesis accompanied reproduction of the bacteria, and that this synthesis was maximal in the *Amylococcus* fraction.

Numerous workers (47, 48, 49) have shown that readily available carbohydrates (starches or sugars) are necessary for the utilization of urea. That conditions *in vitro* are not necessarily a faithful mirror of conditions in the rumen is, however, shown by the investigations of the growth of heifer calves on diets containing urea and extra carbohydrate, carried out by Mills, Lardinois, Rupel & Hart (48). They found that normal growth resulted from a supplement of urea and maize starch but not from one of urea and maize molasses. It still remains to be shown whether the superiority of starch over molasses in animal-feeding experiments with urea is due to the physical or to the chemical nature of the starch, although an obvious explanation seems to be that the sugar may be removed more readily from the sphere of action of the rumen bacteria.

The *general* value of urea as a protein substitute for ruminants does, nevertheless, now appear to be beyond dispute, as will be seen from the following summary of work carried out on sheep (a valuable experimental ruminant on account of its small size and low cost) as well as on cattle.

Johnson, Hamilton, Mitchell & Robinson (49) compared the performance of eight pairs of lambs of equal weight and showed that the utilization of urea was equal to that of soybean meal and superior to that of casein. Harris & Mitchell (50), using ten sheep, showed that the value of urea

for maintenance compared very favourably with that of casein when each of them was used to prevent negative nitrogen balances produced by feeding a ration poor in protein. At equilibrium, however, the casein had a biological value of 79%, while that of urea was only 62%. It was observed that, by sufficient restriction of the casein or urea supplements, biological values of 100% could be demonstrated when the animals were in negative nitrogen balance. The power to utilize urea varied greatly from one animal to another (33, 50). In a subsequent paper Harris & Mitchell (51) investigated the power of urea to promote growth of lambs. They found that a ration containing 11% of conventional protein (N × 6·25), half of which was provided by urea, could maintain over periods of about 100 days a nearly normal growth rate. The biological value of a ration of silage and a carbohydrate supplement containing 5·35% of conventional protein was about 82%. Increasing the urea of the ration by steps up to a concentration equivalent to a ration containing 15% of conventional protein caused a corresponding fall in the biological value of the nitrogen to 44%. Post-mortem examinations of the animals revealed, as might have been expected (Prunty & Roscoe (52)), a hypertrophy of the kidneys at the highest level of urea feeding. Histologically there was no evidence of kidney damage.

Since Hart, Bohstedt, Deobald & Wegner (53) observed kidney damage in the calves to which they had been feeding urea, other workers have sought for further evidence of the nephrotoxicity of urea, and it seems from their observations that the intakes of urea of the calves of Hart *et al.* (53) were needlessly large. In this connexion it should be pointed out that amino-acids may be nephrotoxic if fed in excessive amounts, and that, as Cox, Smythe & Fishback (54) showed, such toxicity may be completely inhibited by the nature of other constituents of the diet. Thus Millar (55), Work, Hamre, Henke & Harris (56), and Harris & Mitchell (51) found by post-mortem examination no evidence of damage to the kidney of sheep or cattle to which urea had been fed. Lenkheit & Schleinitz (57) found a stimulation of the growth of lambs when urea was added to a feed containing potato flakes, while Schmidt & Kliesch (58) confirmed the value of 300 g. urea per head per day using monozygotic twins. Smuts & Marais (59) have shown that negative nitrogen balances in sheep due to paucity in nitrogen of winter grazing in South Africa may be made positive by supplementation of the rations by urea. Klein & Müller (60) showed, in confirmation of their earlier work, that in a ration containing readily available carbohydrate and 80–90% of non-protein nitrogen, positive nitrogen balances, wool growth and increase of weight could be obtained in sheep.

Louw & Van der Wath (61) observed an increase in the numbers of bacteria in the rumen when urea was fed to sheep in a ration similar to that used by Harris & Mitchell (50) and showed that the addition of the urea in this type of ration led to an increase in the digestibility of cellulose from 17·8 to 38·7%. They also found that the addition of either meatmeal or maize and meatmeal to a ration of hay depressed the digestion of cellulose. Progressive supplements of maize in the presence of meatmeal improved the growth of the rumen microflora but did not increase the digestion of cellulose. When either urea or fishmeal was added to a ration containing 0·3% nitrogen, the counts of rumen flora were increased by from 50 to 300%.

Johnson, Hamilton, Robinson & Garey (62) have investigated the nitrogen metabolism and the microbiology of six lambs defaunated by Becker's technique (see later section). The lambs received 83% of their nitrogen intake as urea. With 11·2% crude protein in the diet all six lambs were in positive nitrogen balance, but the retention of nitrogen varied from 4 to 21%. The biological value of the 'protein' in the diet was 45–52 with an average of 49. The apparent digestibility of the nitrogen was 62·8–73·6%. Using rats as the test animals, the biological values of rumen Protozoa and of mixed bacteria from the rumen were compared with that of beef. The true digestibilities of the protozoal and bacterial proteins were 86·2 and 82·4 respectively; their biological values were 68 and 66 respectively. Johnson *et al.* (62) state that their results are consistent with the theory, which has been propounded by Miller & Morrison (63), that provided the nitrogen intake does not exceed the capacity of the micro-organisms of the rumen to utilize it, all the food nitrogen exhibits a biological value characteristic of the mixed micro-organisms reaching the abomasum, a result which is, however, at variance with the earlier findings of

Morris & Wright(64, 65). In this connexion it is interesting to note that the experiments of Loosli & Harris(66) indicate that there may be, from the point of view of biological value, limits to the biosynthetic achievements of ruminal micro-organisms. A further discussion of the whole subject from the point of view of the determination of biological values in ruminants will be found in a paper by Miller & Morrison(67).

Turning to cattle Loosli & McCay(68) have tested the value of urea in feeding six young calves. They found urea was able to supply up to three-quarters of the dietary nitrogen for calves 2 months old. Two of the calves were given, from a few days of age, whole milk and a low-protein basal diet of yellow corn 20 parts, chopped timothy hay 30 parts, corn-starch 37 parts, cane molasses 10 parts, bonemeal 2 parts and salt 1 part. The remaining four calves were fed a similar diet in which 4 parts of urea replaced an equal weight of starch. At about 2 months of age milk was discontinued. Thereafter the calves which were receiving urea gained steadily in weight (61 lb. in 2 months as compared with 80 lb. gained by calves fed a normal ration). The calves deprived of urea gained 7 lb. in 2 months. At 3 months of age the calves were subjected to a 10-day metabolism trial. The four urea calves showed positive nitrogen balances of 11 and 18 g./day, while the two deprived of urea showed balances of 5 and 10 g./day respectively. The added urea produced retentions of 24–36% of the food nitrogen.

Archibald(69), in a 3-year feeding trial extending over two complete lactations and using twenty-eight Holstein cows, has shown that a mixture of urea and corn-starch may successfully replace soyabean and cottonseed meal. Some of Archibald's cows were tested by the double reversal technique with results slightly favouring the ration containing natural protein rather than urea. Two groups of cows were continuously fed throughout two successive lactations, one group on the urea and one group on the control rations. The rates of decline of the milk yield did not differ significantly from one another. Archibald also tested the efficiency of a third ration which resembled the urea ration in every respect but did not contain urea. The omission of the urea caused a serious drop in the milk yield. In these experiments urea formed about 25% of the nitrogen of the entire ration. Mills, Booth, Bohstedt & Hart(70) used a heifer with a rumen fistula to investigate the effect of adding starch to a ration containing urea. Samples were abstracted from the rumen for analysis at intervals after feeding various diets. It was found that ammonia both appeared faster and disappeared faster in the presence of starch, while more protein simultaneously accumulated. Rupel, Bohstedt & Hart(71) compared the milk-producing values of three rations, a basal ration consisting of corn, oats, bonemeal and iodized salt, a normal ration with less corn and oats but linseed meal and ground limestone in place of bonemeal, and an experimental ration of corn, oats, urea, bonemeal and iodized salt. The basal ration contained 10% protein, and the linseed and urea brought the crude protein of the other rations up to 18%. These three rations, with timothy hay and corn silage as roughage, were fed one after the other but not always in the same order. Fifteen cows completed three lactations, one on each of the rations in succession. The lactations on the urea (28·7 lb./day) and linseed rations were comparable and were superior to the lactations on the basal ration (26·1 lb./day). Average gains of weight of 112 and 102 lb. for the fifteen cows were obtained in the linseed and urea lactations respectively against only 25 lb. on the basal ration. The food consumption per 100 lb. of milk (corrected to 4% fat) were almost the same on the urea and the linseed rations, but the consumption of the basal ration was significantly greater. None of the three rations had any significant effect on breeding performance or palatability of milk. There were no significant differences in the urea and total non-protein nitrogen of the bloods of the three groups of cows during the three different dietary regimes. Rupel *et al.*(71) showed, by calculation from prices then ruling in America, that it was profitable to use urea in milk production. With a fourth ration which was fed throughout a lactation to another group of cows, Rupel *et al.* showed that the addition of molasses did not enhance the milk-producing power of urea.

Mills, Lardinois, Rupel & Hart(72), using a fistula animal, found that ammonia concentration resulting from urea feeding fell more rapidly when timothy hay and starch were fed than it did when the starch was omitted. When casein was added as well as starch, hay and urea, the fall

in ammonia concentration was much retarded. These findings are consistent with the view of the authors that the utilization of urea is facilitated by carbohydrate and inhibited by protein. Wegner, Booth, Bohstedt & Hart (73), by determination of true protein and non-protein nitrogen in samples taken from a heifer with a fistula, claim to have demonstrated the synthesis of protein in the rumen, but Pearson & Smith (39) have criticized their deductions because of the difficulty of getting reproducible samples from the rumen. Wegner *et al.* made no incubation *in vitro* to check their deductions.

Briggs, Gallup & Darlow (74) found that four yearling beef steers which were in negative balance on low-grade prairie hay exhibited positive balances when a urea preparation was fed, but not when urea was fed without any other addition to the diet. They also found that two yearling Angus steers fed a fattening ration of grain, hay and 'urea pellets' gained 1·62 lb./head/day over 160 days, while five similar animals fed cottonseed gained over 5 months at a rate of 2·03 lb./head/day.

As regards the methods of feeding synthetic nitrogen compounds to livestock, Millar (55) has developed a process for ammoniating beet pulp to produce a product which does not smell of ammonia. The product when fed to Holstein calves produced growth rates of 1·6 lb./day as compared with 1·96 lb. for a control animal on toasted soyabean meal. Molasses and starch were of equal value in supplementing the ammonia. Millar's animals grew no better on 17% of protein than they did on 12%. Post-mortem examinations revealed no abnormalities in the animals which had received the ammonia.

Many American workers have made successful feeding trials with silage containing urea (75, 76, 77, 78, 79).

Factors affecting the *p*H of the rumen

Many workers have shown that the chief external factors affecting the *p*H of the rumen contents are the lapse of time from the last meal and the kind of food ingested. Thus Phillipson (80) has found that the *p*H of the rumen liquor of sheep fed with either bran plus oats, or mangold plus cabbage, falls from 7 to 6 within the first 4 hr. after eating. He also finds that this fall of *p*H is paralleled by a rise in the concentration of volatile organic acids in the rumen contents. Phillipson also showed that the concentration of lactic acid in the rumen reached a maximum at 1 hr. after feeding and declined to vanishing point before the total volatile acid concentration had reached its maximum. Thus lactic acid could only have contributed to the initial fall in the *p*H. Estimations of the depression of the freezing-point of the rumen liquor showed that the drop in *p*H was accompanied by an increase of osmotic pressure.

In three sheep fasted for 40 hr., the content of volatile acids in the rumen fell progressively, while the *p*H rose to a final figure of 7·5. When hay was fed fermentation was slow and gradual; e.g. in one sheep the *p*H was lowered by only 0·3 of a unit in 6 hr. Production of volatile acids likewise showed only a small and gradual increase which was maximal after 12 hr., while lactic acid production could not be demonstrated. Animals given access to pasture showed a maximal production of volatile acid after 14 hr. While animals were at pasture the concentration of volatile organic acids was always large, and the *p*H was correspondingly lowered. Of the feeds which Phillipson compared, hay produced least and grass most organic acid in the rumen at any one time. Bran-oats mixture and mangold-cabbage mixture were of equal acid-forming power and were intermediate between hay and grass.

These observations of Phillipson are supported by the work of Myburgh & Quin (81) from South Africa, where the constituent ruminal microflora and microfauna may perhaps differ from those in Britain. These investigators found that 4–6 hr. after feeding the *p*H of the rumen became more acid. Lucerne and maize together produced the transient acidity more readily than did maize alone. Acidities ranged from *p*H 5·5 to 6·8. Acid was generated by lucerne hay. However, fresh, green, flowering lucerne or mature veldt-grass hay produced slightly alkaline conditions (*p*H 7·3–7·7). *In vitro* fermentation of glucose by rumen contents led to rapid and definite production of acid and a *p*H of 6·25. Titration of rumen contents with either normal hydrochloric

acid or normal sodium hydroxide showed that they were well buffered. 150 ml. of normal hydrochloric acid added through a fistula reduced the *p*H of the rumen to 5·9. The colour, odour and power to ferment glucose varied with the *p*H of the rumen.

That the *p*H of the rumen contents of cattle varies between much the same limits as are found for sheep (*p*H 6–7·14)(82, 83, 84) has been shown by various American workers. There is, however, a difference of opinion as to the substrate from which these organic acids are derived *in vivo*. There is an even greater difference of opinion as to what particular micro-organisms in the rumen are chiefly responsible for their production. The work of Van der Wath & Myburgh(85) shows, however, that the multiplication of the larger infusoria of the rumen is inhibited by a *p*H below 6, such as is produced by feeding a diet of yellow maize alone. Since starchy foods, as shown by these authors and by Phillipson(80), lead to a rapid production of acid it would seem that starches are potent sources of acids. Sugars are also potent acid producers in sheep which harbour *Schizosaccharomyces ovis* Quin(86), so much so that their presence generates from the bicarbonate of the saliva large volumes of carbon dioxide, a fact which may be of significance in the etiology of bloat due to sudden access to luscious young lucerne pastures(87).

Ammonia produced from non-protein nitrogen, which forms over 40% of the nitrogen of many vegetables(88), will neutralize organic acids simultaneously formed. This phenomenon of self-neutralization also occurs in silage (unpublished observations of the author). Proteolysis which readily takes place in the rumen(39) will likewise provide ammonia for neutralization of the organic acids. Evidently organic acids are formed in the rumen from the many foods in concentrations greater than can be neutralized by ammonia production. The third and probably the most potent agency helping to maintain the *p*H of the rumen is the copious secretion of the saliva which is alkaline and of great buffering power owing to its richness in phosphate.

A fourth factor which probably assists in maintaining the *p*H of the rumen has been studied by Danielli, Hitchcock, Marshall & Phillipson(89), who showed that the permeability of the epithelium which lines the rumen varies with *p*H. At *p*H 7·5 acetic, propionic and butyric acids will be present as ions. These ions were shown to be absorbed through the ruminal epithelium together with an equivalent amount of kation, so that the absorption had little effect on the *p*H. At *p*H 5·8, however, the free acids passed through the epithelium more rapidly than the anions, thus tending to drive the *p*H towards neutrality. The authors state that the rates of loss from the rumen due to absorption through its wall are such that at *p*H 7·5, acetate, propionate and butyrate are absorbed at rates which decrease with ionic weight (as would be expected by Graham's law), whereas at *p*H 5·8 absorption is much faster for all the acids and the rates of absorption increase with molecular weight, i.e. with increasing lipoid solubility. An investigation of the permeability of the rumen to such substances as ammonia, urea, and asparagine would be of interest, but Danielli *et al.* did not study that phase of rumen physiology.

Carbon dioxide which is continuously produced in the rumen as a result of the neutralization of fatty acids by bicarbonate in the saliva probably gives rise to methane as a result of reduction by rumen bacteria(18).

The etiology of bloat

Reference has already been made to the possible significance of *Schizosaccharomyces ovis* Quin, present in the rumen of South African sheep, in the etiology of bloat. Working with sheep as experimental animals Clark & Quin(90) have investigated the toxicity of potassium cyanide introduced into the rumen through a fistula. Sheep fed as little as a pound of lucerne hay could tolerate doses of 650–1000 mg. KCN before the rumen became paralysed.

By contrast 175 mg. KCN was sufficient to paralyse the rumen in sheep starved for 14 hr. The effect of the lucerne was found to be due to an increase in the rate of respiration as a result of the carbon dioxide produced by fermentation of the lucerne, for bubbling CO_2 from a cylinder through the rumen contents had the same effect. Even when the rumen was paralysed eructation still took place, the sheep being able to belch every hour 2 l. of gas introduced through the fistula. The authors conclude from these experiments that the inability to eructate is not necessarily

engendered by ruminal paralysis, and that the effect of roughage in preventing bloat may be a purely mechanical rather than a reflex action. They also conclude that cyanogenetic factors in the food are not agents in the production of bloat.

Kleiber, Cole & Mead (91) analysed rumen gases from bloated cattle and found 67% carbon dioxide, 26% methane, less than 1% oxygen and 0·11% hydrogen sulphide. They believe that bloat is caused by inability to belch and not by abnormal gas formation. Mead, Cole & Regan (92) increased the incidence of bloat by grinding the roughage which consisted of alfalfa hay.

Although bloat may be prevented by feeding roughage before allowing animals to have access to fresh young pasture, its etiology still remains obscure. For a recent discussion of the subject the reader is referred to the review by Cole, Huffman, Kleiber, Olson & Schalk (93).

The work of Clark & Quin (90) has shown how the rumen can *reduce* the toxicity of cyanide. It is therefore pertinent to allude at this point to a toxicosis of cattle in which toxicity of ingesta is *increased* by the effect of the rumen contents on it. What has been called 'oat-hay poisoning' occurs when cattle have access to certain kinds of spoiled hay or to pastures recently dressed with nitrate or to freshly dug mangolds. In this disease the substance ingested, i.e. nitrate, is not in itself toxic. Toxicity is engendered by the reduction of the nitrate to nitrite in the rumen. The nitrite presumably interferes with the animal's health by combining with haemoglobin.

The importance of the microflora and microfauna in the physiology of the rumen

Reference has already been made to the role of ruminant microflora in the utilization of urea. Certain work on the more general aspects of ruminant micro-organisms should, however, be included in this review.

The paunch and reticulum of the sheep, goat and cow, the caecum of the horse, pig, guinea-pig, rabbit and rat (94), and the paunch of the camel, deer, and of various species of African antelope, are sites in the gut where the passage of food is delayed. The large intestine of the primates including man is also of a like nature. In these special parts of the digestive tract bacteria abound and many species and morphological types are present (94, 95, 96). In the ruminants the paunch, and in the horse the caecum, contain, in addition, numerous Protozoa allied to the common hay animalcule *Paramecium* but of a definite variety or species peculiar to their respective hosts. Morphologically, these ruminal Infusoria have been extensively investigated (97).

In both domesticated and wild ruminants the Infusoria are particularly sensitive to changes in the diet (85). Their numbers wane when food is withheld or when maize, which produces a low *p*H in the rumen, is fed. Becker (98) and his colleagues devised a technique for freeing ruminants of their ruminal Infusoria. They subjected sheep to several days of starvation followed by one or more drenchings with a 2% solution of copper sulphate before refeeding. Under these circumstances abstraction of rumen contents by means of a stomach pump showed that the animals remained free of Infusoria so long as they were isolated. Even the feeding of unsterilized hay, contrary to what might have been expected from the observations of Fantham (99), failed to reinstate the Infusoria. Becker & Everett (100) found that such animals, far from showing any signs of ill-health, gained weight slightly faster than untreated controls.

Van der Wath & Myburgh (85), using Becker's technique, defaunated sheep and investigated the rate of disappearance of starch from the rumen contents. They reinfected the rumen contents with an iodophilic coccus which the defaunation had also killed. Starch disappeared just as rapidly from the rumen contents of defaunates as from untreated animals. Unfortunately, the same authors do not record what happened when starch was fed to defaunated animals in the absence of the iodophilic coccus. They could not demonstrate any effect of defaunation on the animal's power to digest cellulose, and thus gave weight to Mangold's theory, also supported by Baker (94), that digestion of cellulose in the rumen is entirely due to bacteria, and that solution of pieces of vegetable matter ingested by Infusoria, which many microscopists have observed, is due to bacteria adherent to the surface of such vegetable matter. Digestion of starch within the body cavities of the rumen Infusoria is rapid and com-

plete, but, on the basis of the results of Van der Wath & Myburgh already quoted, must be presumed to be due—like that of cellulose—to adherent bacteria, since even in normal animals starch which is free in the rumen disappears as quickly as starch within the Protozoa (85). Nevertheless, Hungate (101, 102), by growing isolated species of ruminal Infusoria *in vitro*, claims to have demonstrated that species of *Diplodinium* digest cellulose while *Entodinium caudatum* does not.

Van der Wath & Myburgh (85) also observed that as starch disappeared from the body cavities of the Infusoria a material staining brown with iodine appeared in the infusorial cytoplasm. This material has usually been called infusorial glycogen (103). They believe, however, that these so-called glycogen inclusions are really food vacuoles containing bacteria undergoing phagocytosis, the brown stain with iodine being due to a bacterial polysaccharide of the glycogen type, stored by the bacteria when they were digesting the starch within the body cavity of the infusorian. Further observations on cattle would throw light on this point, for rumen bacteria in cattle build up bacterial starch (94).

Many attempts have been made to elucidate the microbiological changes occurring in the rumen by isolating organisms from the rumen and growing them in pure culture. An organism, *Plectridium cellulolyticum*, was isolated from the sheep's rumen by Pochon (104). This organism in suitable media fermented cellulose, provided in the form of filter paper, and formic, acetic and propionic acids, and ethanol accumulated in the medium. In the medium, reducing sugars could not be demonstrated, but when cellulose was incubated with cultures sterilized by toluene, reducing sugars accumulated. From the sterilized cultures glucosazone was prepared and identified. A second crystalline osazone was assumed, but without proof, to be cellobiosazone. Gas was also produced but not identified. Pochon's medium was a heat-sterilized filtrate of the rumen contents of cattle brought to *p*H 7·5–8 by means of calcium carbonate, having filter-paper added to indicate cellulolysis, and rendered anaerobic by covering with a layer of mineral oil. Numerous other media were also tried, but no success resulted from their use. There is great difficulty in applying Pochon's results to elucidate the fermentation in the rumen. A *p*H which is on the alkaline side of neutrality, even if calcium carbonate had not been added, is unusual, and Pochon does not specifically state the kind and condition of the animal from which the organism *P. cellulolyticum* with which he worked was obtained. He states, however, that his organism arose as a single colony isolated by subculture of sporeformers. Thus there is considerable risk that it may have been a mere passenger in the rumen.

Elsden (105) found that the main volatile acids produced in the sheep's rumen were acetic, propionic and butyric, acetic forming from a half to three-quarters of the total. When cellulose, glucose, or lactic acid was subjected *in vitro* to the action of rumen contents the same three acids were produced, with propionic acid preponderating. Little butyric acid was produced when the main substrate was cellulose. *In vitro* and *in vivo* glucose exhibited similar fermentations. When dried grass was incubated with rumen contents *in vitro* acetic acid preponderated. Elsden isolated from the rumen a *Propionibacterium* and presented evidence that it was responsible for the production of propionic acid in the rumen. He also showed that the animal's dietary history affected the rate of fermentation of glucose in the rumen and that the composition of the microflora was affected by the diet.

Johnson *et al.* (62) isolated what they called a *Flavobacterium* from rumen contents. It was an aerobic organism but was not further specified or characterized. Counts of Protozoa, bacteria and a schizomycete (unidentified) were also made. On the urea diet which they fed, methane production was related to bacterial counts. Hamilton (106) found that the addition of glucose to the rations diminished the digestibility of cellulose in the diet and suggested that this was because the rumen bacteria preferred glucose to cellulose as a substrate.

McNaught & Smith (107) found that the carbon of maltose gave rise when incubated with rumen liquor to bacterial polysaccharide 14·7%, volatile fatty acids 34·7%, lactic acid 8·5%, carbon dioxide 13·7%, methane 4·2% and protein 13·8%, so that 89·6% of the carbon in the maltose was accounted for.

McAnally(5) investigated the digestion of straw and other sources of cellulose by suspending them in the rumen in silk bags. She compared the rate of digestion of prepared cellulose, of oat straw, of wheat straw, and of undigested residues left in various parts of the gut of the sheep fed on oat or wheat straws. Finding, contrary to Trautmann & Asher(108), a lower percentage of cellulose in faecal residues than in residues from the rumen or abomasum, she concluded that cellulose digestion is continued in the post-abomasal regions of the gut. The redigestion in the rumen of washed faecal residues demonstrates the presence in them of a 'hard core' of indigestible polysaccharide forming 35% of such faecal residues. McAnally further found that all the three main fractions of straw, namely, cellulose, pentosan-polyuronide, and lignin, are more readily digested *in vivo* and *in vitro* by rumen micro-organisms when isolated than when present together in their natural state in straw. Lignin, contrary to the assumptions of those who have attempted to determine digestibilities in ruminants(109) by means of lignin ratios, is partly lost during the digestion of straw either *in vivo* or *in vitro*. McAnally(110) had earlier found that sugars when added to rumen liquor from merino sheep were attacked in the following order: glucose, sucrose, maltose, cellobiose and lactose. The last would not normally occur in the food of adult sheep, whereas the first two would. Maltose and cellobiose were included because they were degradation products of starch and cellulose respectively.

Claren(111) found that ingested formate rapidly disappeared from the rumen without appearing in urine or faeces, even after 10 days. He therefore assumed that it was rapidly changed by the rumen flora. He found 0·02–0·04% formic acid to be normally present in the sheep's rumen. Each of three sheep ingested 20 g. formic acid, half-neutralized with NH_3, prior to slaughter and immediate post-mortem ligaturing of various sections of the gut. In no part of the gut could formic acid be demonstrated. Bacteria which he isolated from the rumen caused the rapid disappearance of formic acid. Carbon dioxide was produced, but no increase in hydrogen could be demonstrated. He does not specify the types of bacteria isolated or how he isolated them.

Proteins and amino-acids

Inchausti, Tagle & Helman(112) found no effect on the yield or composition of milk as a result of feeding groups of cattle on rations of different nutritive ratios, but the cows receiving more protein showed greater increases in weight. Miller & Morrison(113) observed that the protein of soyabeans had the same digestibility irrespective of the heat treatment or oil extraction process to which the beans had been subjected. Franke(114) found no difference in a 3-months' experiment between rations containing protein of plant origin and rations containing animal protein. Laptev(115) found that spermatogenesis in bulls was more favourably affected by bloodmeal than by skim milk. Concentrates of plant origin were less effective than skim milk. Braude(116) has reviewed the literature on yeast in the rations of cattle and pigs.

Kehar, Muckherjee & Sen(117), by feeding bullocks on a diet containing only 0·02 g. N/100 g. dry matter, calculated that their endogenous urinary nitrogen was 0·02 g./kg. body weight. Kehar, Muckherjee, Murty & Sen(118) found that such bullocks exhibited no change of excretion of urinary nitrogen, creatine, creatinine, total sulphur or total sulphate as a result of the muscular work involved in traction, provided they were given extra carbohydrate to cover their extra energy expenditure.

Shrewsbury, Andrews, Harper & Zelle(119) found that deficiencies in a diet of oat straw, maize and maize silage fed to breeding ewes could be made good by addition of extra protein, vitamin A and a water-soluble fraction from lucerne juice. A useful review of the amino-acid content of pasture proteins has been written by Lugg(120). The biological value of protein and non-protein nitrogen has been dealt with in an earlier section.

METABOLISM OF DIETARY FAT AND ETHER EXTRACT

Rations containing 2·6% ether extract were found by Lucas & Loosli(121) to have the same digestibility of proximate constituents as rations containing 1·6%, but rations containing 1% ether extract showed better digestibilities of crude fibre and nitrogen-free extractives than the same rations fortified to 7% ether extract by additions of corn oil or soyabean oil or fatty acids of soyabean oil. Added oil or fatty acids depressed milk yield without affecting the percentage of milk fat. This report apparently conflicts with numerous earlier reports of Maynard and his associates. Thus Maynard, Loosli & McCay(122) found significantly greater milk and fat yields from cows receiving 7% fat in their concentrates than from those receiving only 3%. The explanation of this conflict of results is to be found in a paper by Loosli, Maynard & Lucas(123) confirming the earlier results of Maynard and his associates but showing that the effects of fat were modified by the composition of the remainder of the diet. The same workers in another paper(124) show that large reductions of roughage intake cause large reductions of fat percentage in milk.

Hilditch & Jasperson(125) compared the composition of composite samples of butter produced from five groups of three cows each. Group I received a normal ration, groups II, III and IV the same ration plus peanut oil, and group V the same ration plus palm-kernel oil of iodine value 17. Refined peanut oil of iodine value 88 was fed to group II, hydrogenated peanut oil of iodine value 45 to group III and hydrogenated peanut oil of iodine value 17 to group IV. The analysis by ester fractionation of the resulting butter fat showed that the chemical composition of the fats fed was to some extent reflected in that of milk fats produced. The completely hydrogenated oil gave a milk fat almost identical with that given by the control rations, except for a slight increase of oleic and stearic acids. According to the authors, this is due to the presence in the fat of glycerides such as tristearin, the higher melting-point of which tends to reduce their rate of assimilation. No analysis of faecal fats was, however, undertaken to check this hypothesis. This experiment, when considered in conjunction with earlier work quoted by Hilditch & Jasperson(125), supports the hypothesis that milk fat originates from glycerides circulating in the blood. However, this hypothesis, though necessary to explain the secretion of milk fat, is not sufficient, especially when the lower fatty acids are under consideration. The origin of the lower fatty acids is still being actively investigated. Thus Kaufman & Shaw(126) found, as had Smith & Dastur(127) before them, that the Reichert-Meissl value of milk fat was decreased by starvation, while when carbohydrate alone was fed the synthesis of lower fatty acids in milk was enhanced. The fact that lower fatty acids are important products of ruminant digestion and that they pass into the blood as such, probably explains these observations of Kaufman & Shaw. Hilditch & Meara(128) advance the hypothesis that the lower fatty acids in milk arise by rupture of longer-chain acids containing ethenoid linkages, e.g. oleic and linoleic acids which are abundant in the fat of grass(129).

Shaw, Powell & Knodt(130) state that in the hypoglycaemic cow the secretion of lower fatty acids in milk fat of cows is not diminished. This would tend to reduce the probability of the hypothesis that lower fatty acids are formed from glucose, and, if we assume that the higher fatty acids come from arterial mammary blood preformed, we are left without any explanation of Shaw's observation(131) that the R.Q. of the actively functioning mammary gland is greater than unity. Shaw *et al.*(130) suggest that, since the concentration of lower fatty acids is more closely related to food intake than to glucose, to lactic acid or to ketone bodies in the blood, the rumen may play a part in their production. Recent work from Cambridge(105) on the amounts of lower fatty acids, particularly of acetic, generated by the micro-organisms present in the rumen, make this suggestion well worth investigation. Such a hypothesis helps to explain the table presented by Hilditch(132) of the Reichert-Meissl values of the fats of the milks of various mammals. Hilditch has arranged these species in order of increasing Reichert-Meissl value. It so happens, however (though Hilditch does not point this out), that in the list the non-herbivorous species, dog, pig, man, mouse and cat, come first, the herbivorous non-ruminants,

horse, ass and rabbit, come next and the ruminants, camel, goat, sheep, buffalo and cow, come last with the highest values. When it is realized how sensitive the production of lower fatty acids in the rumen is to dietary changes and that in sheep their production is great when grass is fed (105), the hypothesis that the lower fatty acids are microbiological in origin is indeed tempting. Recent work by Hilditch & Meara (128), on the composition of the fat of human milk and of Hilditch & Jasperson (125) on the fat of the milks of the goat, the ewe and the mare, gives figures which fall in the expected places in Hilditch's earlier published table of species differences (132).

There has been a renewal of interest in both the anabolism and catabolism of acetic and other lower fatty acids, due partly to the availability of heavy and of radioactive isotopes in America. Thus Bloch & Rittenberg (133) found that acetate but not propionate or butyrate or other higher fatty acid forms cholesterol in the body. Buchanan, Hastings & Nesbett (134), however, found that, of fatty acids containing radio-carbon, propionic and butyric but not acetic when fed to rats could help to form glycogen in the liver. Radio-carbon from acetic acid could not be detected in liver fat. By feeding experiments McManus, Bender & Garrett (135) found that acetic acid fed in the form of glycerol triacetate had the same calorie value as glucose, and McClendon & Scott (136) found that acetic acid was readily absorbed by the portal system and was removed from the blood by the liver. Rittenberg & Bloch (137), using deuterium and heavy carbon, have demonstrated that in contrast to the reports of Buchanan *et al.* (134) both the methyl and carbonyl carbons of acetic acid can be precursors of carbon in carcass and liver fats and that fat is probably formed by successive condensation of C_2 units. Medes, Floyd & Weinhouse (138) found acetic acid to be oxidized by tissues with the intermediate formation of acetoacetic acid which, however, accumulated only in the liver. Bloch & Rittenberg (139) have presented evidence suggesting β-oxidation of fat as the chief source of acetic acid in the body of the rat.

Powell (140) has presented data from ninety-nine lactations of fifty-one cows to support his conclusions that when roughage is reduced to 6 lb. of alfalfa per head per day and concentrates are fed to the full allowance, milk fat is decreased as long as the roughage reduction continues. When the concentrates were fermented either alone or with addition of rumen contents from slaughtered animals to which normal diets had been fed, average milk fat increased by 1%. Butyric acid and sugar had no such effect. Unfortunately, neither acetic nor propionic acids were tested, although the work of Elsden (44, 105) shows that these acids are produced far more abundantly than butyric in the rumen.

The mode of administration of oils to calves may be important. Thus Bate, Espe & Cannon (141) found that alopecia of the perineal region and of the inner surfaces of the thighs occurred in calves given skim milk and 2% of soyabean oil. 3·3% of the oil produced scours. On the 2% fat-skim milk diet the calves thrived but were subnormal and subject to steatorrhoea. The untoward effects of the oil could be prevented in older calves by allowing access to grain or pasture. When access to pasture or grain was not allowed the alopecia could be cured by homogenizing the skim milk and the fat, although relapse occurred when the homogenization was discontinued.

That emulsification has important effects in facilitating absorption even of hydrocarbons is shown by the experiments of Frazer, Stewart & Schulman on paraffin oil (142), and the observation of Kon, Mawson & Thompson (143) may indicate a special natural provision for efficient absorption of carotene.

Vitamin A and carotene

By investigation of the seasonal and geographical distribution of acetonemia in American cattle Patton (144) has concluded that this ailment is correlated with absence of fresh pasture for a large period of the year with a consequently diminished intake of carotene. Patton claims that such acetonemia is curable either by vitamin A or by good feeding practices. Sisk (145) reports that acetonaemia of cattle responds better to dextrose and vitamin A than to dextrose alone. Burt (146) also reports success with vitamin A. Contrary to Sisk and Patton are the findings of Hayden, Fincher, Roberts, Gibbons & Danks (147) that acetonaemia in cattle is not cured by vitamin A,

and the observation of Underwood, Curnow & Shier(148) that the livers of toxaemic pregnant ewes contained 391 i.u. vitamin A/g., while livers of foetuses of both normal and toxaemic ewes contained only traces.

Seshan & Sen(149) have carried out carotene balance experiments on cows and bullocks and have shown that the non-appearance of so much of ingested carotene in butterfat is largely due to the great wastage of β-carotene in the faeces. Even when the intake of carotene is only 7–10 mg., most of it is wasted in the faeces. Though both retention of carotene and blood carotene increase with increasing intake from green berseem the faecal output may be taken as a good indication of the carotene intake. After single large feeds of green berseem about 5 days is required before the faecal excretion returns to its initial value, showing presumably that the gut takes at least 5 days to empty itself and that carotene is stable while passing through the gut. The bovine does not absorb carotene readily, but the cause of this difficulty of absorption has not been investigated. In a later paper Sarkar & Sen(150) noted that increase of intake of green stuff above 60 lb. caused no further increase of the vitamin A potency of milk fat. When hay supplying 24,000 i.u. was changed to 60 lb. greenstuff supplying 3,000,000 i.u., the total potency of milk fat increased from 6000 to 10,000 i.u./lb., the percentage increase of carotene being many times the increase of A. In these experiments increase of greenstuff appeared to increase the unsaturation of the milk fat. The transfer of carotene from greenstuff to milk was only 0·5%. While receiving hay the cows were in negative carotene balance, but from greenstuff 30% of the carotene was absorbed and positive balances resulted. Carotene and vitamin A in blood plasma increased after greenstuff. Similar observations were made whether green berseem, green oat grass, or green dub grass, *Cynodon dactylon*, were used. The same authors(151) found that the vitamin A potency of green fodder is half of its β-carotene content.

Moore & Berry(152) estimated every fortnight the vitamin A and carotene in the plasma of the blood of three groups of calves, until the animals were 4 months old. One group was deprived of carotene or other source of vitamin A. The second group received hay made from clover and timothy containing 15 p.p.m. carotene. The third group received lespedeza hay containing 50 p.p.m. carotene. Both clover-timothy and lespedeza hays enabled the calves to increase the vitamin A in blood plasma as they grew. Of six calves getting clover-timothy hay the vitamin A in the blood plasma ranged from 7·5 to 10·5 μg./100 ml. after 90 days. For three calves getting lespedeza the corresponding values were 22·7–27·8. All nine calves were then deprived of carotene and at 126–128 days of age the blood plasma of the clover-timothy group showed 0·5–5·1 μg./100 ml. vitamin A while the lespedeza group showed the much larger figures 6·6–13·3. From these results Moore & Berry conclude that the lespedeza hay produced the larger reserve of vitamin A in the liver.

Kuhlman & Gallup(153) have shown the importance of hay as a source of carotene to the young calf and to the growing bovine by replacing part of the hay of the ration by a carotene concentrate and demonstrating satisfactory growth in calves so treated. They could not demonstrate any carotene in the plasma of the blood of the new-born calf, but it rapidly increased with increasing intake. Growth was satisfactory when the carotene intake was 22–180 μg./lb. bodyweight/day. Kuhlman & Gallup also found that if at 18–24 months of age the animal's blood plasma contained less than 150 μg./100 ml., difficulties were likely to be encountered at subsequent parturition or at the inception of lactation in spite of the apparently normal physical appearance of the animals.

Boyer, Phillips, Pounden, Jensen, Rupel & Nesbit(154) produced acute deficienty of vitamin A in eleven calves, and found that with the reduction of intake of the vitamin, there occurred a reduction of vitamin A and of ascorbic acid in the blood plasma, and that the increase of pressure of the cerebrospinal fluid was accompanied by a decrease of ascorbic acid therein. From rat experiments carried out at the same time they inferred that the reduction of the ascorbic acid in blood and in cerebrospinal fluid was not due to increased excretion of ascorbic acid but to defective synthesis. Observations on the effect of ascorbic acid in reducing the intracranial pressure were conflicting. In both normal and vitamin A-deficient calves the concentration of ascorbic acid in cerebrospinal fluid was some six times that of the blood plasma. In three out of

five vitamin A-deficient calves the administration of ascorbic acid raised the ascorbic acid in the cerebrospinal fluid and reduced the intracranial pressure. Moore(155) has investigated the suggestion of Boyer *et al.*(154) that defective synthesis of ascorbic acid plays a part in the reduction of intracranial pressure resulting when calves are deprived of carotene and vitamin A. Moore found that when normal values of ascorbic acid in the blood were maintained whether by feeding chlorbutanol or by injecting ascorbic acid, carotene deprivation still caused a rise of intracranial pressure. He also showed that blindness due to optic stenosis resulting from carotene deficiency was not prevented by ascorbic acid injections. As a result of these findings Moore rejects the suggestion of Boyer *et al.*(154) that ascorbic acid influences intracranial pressure.

Moore, Berry & Sykes(156) have further reported that intracranial pressure of calves is a fairly sensitive indicator of their carotene intake. By this criterion calves require 66 μg. carotene/kg. body weight. Moore *et al.* could not confirm the observations of Boyer *et al.*(154) that deficiency of carotene induces deficiency of ascorbic acid in calves. They say, however, that the deficiencies of their calves were not so severe as these of Boyer *et al.*(154).

Lundquist & Phillips(157), working with an unspecified number of calves, report that calf survival is higher after treatment with vitamin A than it is after carotene, and that neither A nor carotene affects the ascorbic acid content of the blood plasma. The same authors(158) claim that young calves, though born with adequate ascorbic acid in the blood stream soon exhaust the supply, and if fed skim milk are benefited by the oral administration of ascorbic acid after the 10th day of life. Two to three weeks later the calves are able to cover their requirement of ascorbic acid by synthesis. With supplements of ascorbic acid, vitamins A and D and nicotinic acid, Holstein calves were able to survive on skim milk. As a remedy for scours pantothenic acid was ineffective. Guernsey and Jersey calves did not survive unless they were nursed by their dams for 2 or 3 days. Carotene in oil failed to raise the low level of A in the plasma of young calves.

In contrast to the findings of Lundquist & Phillips(158) Norton, Easton, Loosli & Spielman(159), using sixty Holstein heifer calves, found that those receiving capsules containing vitamins A, D, E, ascorbic acid, aneurin, riboflavin, panthothenic acid, nicotinic acid and choline were no healthier than untreated controls. The authors say, however, that the basal diet used may have been better than that of Lundquist & Phillips(158).

Moore & Cotter(160) found that restriction of the carotene intake of pregnant dairy heifers, mostly Holsteins, led to an increase of 4–6 times in the excretion of ascorbic acid in the urine. Daily administration of 5 g. chlorbutanol had the same effect. Combination of both treatments had little further effect on ascorbic acid excretion.

Lucas, Loosli & Maynard(161) have reported that supplements of vitamin A, while not affecting the yield of milk were able to raise the A in blood and in milk fat by 25 and 70% respectively. Variations of A intake were without effect on blood ascorbic acid.

Erb, Andrews, Bullard & Hilton(162) have described biopsies of degeneration in the seminiferous tubules of a vitamin A-deficient bull which also exhibited as symptoms, blindness, muscular inco-ordination, bloat and diarrhoea. It took 6 months for this condition to be reached after cod-liver oil was withheld. After 4 months of vitamin A therapy a second biopsy revealed that the testicle was again becoming normal, but it was much longer before sperm secretion became normal.

To prevent obvious symptoms of vitamin A-deficiency, i.e. arrest of growth, blindness, diarrhoea and impotence in dairy bulls, Jones & Haag(163) found 65 μg. carotene/kg. body weight to be sufficient. At 5, 20 or 35 μg. growth was arrested. Night blindness developed in 45–268 days (depending upon age at beginning of the experiment) in steer calves deprived of carotene in an investigation by Jones, Schmidt, Dickson, Fraps, Jones, Riggs, Kemmerer, Howe, Black, Ellis & Marion(164), who found that 2500 μg. failed to prevent increase of night blindness, but 3000–5000 μg. led to improvement. After depletion satisfactory fattening could be achieved by feeding 800–1250 μg., but for complete well-being 2000–2500 μg. were recommended. Jones *et al.*(164) found night blindness to be the first sign of carotene deficiency. They record the vitamin A and carotene contents in Texas feeds and in the blood and liver of experimental steers.

In spite of the occurrence of night blindness in many species as a result of deficiency of vitamin A, Morton (165) has questioned Wald's theory that visual purple is a complex of vitamin A and protein and has drawn attention to earlier work on riboflavin which is also abundant in the retina.

Boyer, Phillips, Lundquist, Jensen & Rupel (166), in a long-term experiment, found that vitamin A in the blood plasma was a better indicator of vitamin A intake than either plasma carotene or growth. On intakes which were just adequate calves had 10 μg./100 ml. plasma. Values of 7–8 μg. indicated an inadequate intake. Intakes of 6, 12 and 18 μg./kg. body weight maintained, respectively, inadequate, borderline and adequate concentrations of vitamin A in the blood plasma. Adequate intakes of carotene were higher, i.e. 75–125 μg./kg.

Tarassuk & Regan (167) maintained six cows for periods up to 269 days on bleached alfalfa hay and concentrates. Carotene in the blood plasma was reduced from 2·5 to 0·25 mg./100 ml. At the same time the lipolytic activity of the milk was much increased as measured by the fall in surface tension resulting from hydrolytic rancidity developed during storage. Daily administration of 1 g. carotene in oil produced normal blood carotene, but had no effect on the lipolysis which was, however, reduced to normal by the feeding of greenstuff.

Keener, Bechdel, Guerrant & Thorp (168), in producing vitamin A deficiency in thirty-four calves by means of a diet poor in carotene, claim to have shown that the requirement of carotene is much larger when the weather is colder. They state that the minimum carotene requirement of calves to prevent histologically demonstrable changes is 26 μg./kg. body weight when the temperature is 60–70° F.

Madsen, Hall & Converse (169) observed a hitherto unreported effect of vitamin A deficiency in six out of seven young cattle aged up to 2 years whose dams had received diets poor in carotene and in seven out of eight calves weaned on to diet poor in carotene. The animal's pituitary bodies were cystic. Other more usually recorded effects, e.g. retarded growth, papilloedema and blindness were also observed. The cystic pituitaries were not corrected by carotene in oil. In 10,000 slaughterhouse samples the incidence of cystic pituitary was only 0·005%.

Braun & Cable (170) compared the concentrations of vitamin A in the livers of dam and of foetus aborted by brucellosis. The A in the foetal liver reflected the A in the dam's liver which in turn reflected the dietary A.

Wendt (171) has reported the development by heifers of an appetite for conifer shoots during a shortage of hay in winter. The desire was not evident in cows injected with vitamin A.

Rusoff, Skipper & Arnold (172) fed shark-liver oil to Jersey cows at rates of 2·5, 5 and 10 lb./ton of mixed concentrates which were fed with silage and clover hay. Intakes of vitamin A of cows varied from three-quarters to over two million i.u. for short periods. Large intakes did not reduce the yields of milk but had a slightly depressing effect on the fat percentages. Maximal potency of the milk was about 2000 i.u./qt. and was not increased by intakes in excess of three-quarters of a million i.u. Milk had 1570 i.u./qt. on the unsupplemented ration. Ascorbic acid in the milk was unaffected by the extra intake of vitamin A. Too much oil at one time probably prevents adequate emulsification and produces steatorrhoea. Thus Moore, Hoffman & Berry (173) noticed that fat percentage was reduced by 5–7 oz. of cod-liver oil in a single dose each day, while at the same time the iodine value of the fat increased. When, however, the oil was given in twelve separate doses these effects were much less marked. The quantities fed by Moore *et al.* are needlessly high for producing maximal vitamin A in the milk as calculations from the results of Rusoff *et al.* (172) show.

Rupel, Boyer & Phillips (174) found that 90 ml., but not 25 ml., of shark-liver oil per day depressed the yields of cow's milk after 8 weeks without affecting its flavour. Jensen, Boyer, Phillips, Rupel & Lundquist (175) state that 1½ million i.u. of A per head per day as shark-liver oil had no effect on milk or fat production. The vitamin A content increased and the carotene decreased in both milk and blood. These large doses of vitamin A had no effect on the ascorbic acid in the blood plasma. Similar results using Holstein and Jersey cows are reported by Fontaine & Bolin (176), who fed 60 ml./head/day of a shark-liver oil containing 25,000 i.u./g., the basal

ration like those in the other experiments cited in this regard being adequate in carotene. No effect on yield of either milk or fat was observed in either breed of cow, but the vitamin A of the milk in both breeds was increased four times while carotene decreased by 22% in Holsteins and 33% in Jerseys. Deuel, Hallman, Johnston & Mattson (177) have found that the lowering of the carotene concentration in milk and in blood which occurs when 700,000–2,100,000 i.u. of vitamin A are fed to Guernsey and Holstein cows is due to the vitamin A itself and not to other constituents of the high-potency shark-liver oil used. The shark-liver oil did not have the depressing effect on milk yield recorded by Golding, Soames & Zilva (178) for cod-liver oil. The intensive feeding of vitamin A either as concentrate or as shark-liver oil caused an increase in the vitamin A in blood and in milk, though the extra vitamin A in the milk was only 2–4% of that fed. The effect of shark-liver oil on milk yield and on carotene and vitamin A in milk has also been investigated by Blaxter, Kon & Thompson (179).

Other fat-soluble vitamins

Vitamin D. Recent experiments using radioactive calcium confirm earlier theories that the physiological effect of vitamin D is to favour intestinal absorption and mineralization of bone (180). Presumably owing to the greater difficulty of determining vitamin D than vitamin A no attention has been paid in experiments with fish-liver oil, already cited, to possible effects of the vitamin D in the oils, on the physiology of the cow or the D-potency of its milk.

Gallup & Kuhlman (181) have shown that Oklahoma sunshine is sufficient to protect calves from rickets due to rations poor in vitamin D, though direct exposure to sunshine was necessary, mere daylight being inadequate. Allemann (182) has shown the importance of sunshine in developing vitamin D-activity in the drying of green stuffs on the farm.

Vitamin E (*tocopherols*). Deficiency of vitamin E produces symptoms which vary with age, sex and species and with the composition of the basal diet (183). It is not surprising then to find that observations on cattle do not lead to the same conclusions as those on sheep. Thus Gullickson, Palmer, Boyd & Olson (184) reared and bred fifteen cows and seven bulls on diets free from vitamin E without any obvious untoward effects. In only two cases was more than one service required for conception. Gullickson *et al.* found vitamin E in the faeces of cattle on normal rations but none in the faeces of the vitamin E-deficient animals. Neither could they detect the vitamin in the tissues of animals not receiving it. They therefore concluded that cattle do not need vitamin E. This experiment recalls to mind the similar results obtained with goats by Thomas, Cannon, McNutt & Underbjerg (185).

By contrast, Marston & Pierce (186) produced muscular dystrophy in sheep kept for 3 years on a synthetic diet, which they suggest was due to vitamin E-deficiency, while Willmann, Loosli, Asdell, Morrison & Olafson (187), in an investigation of 'stiff-lamb' disease, were able to prevent the typical symptoms by the administration of *dl*-α-tocopherol.

Vitamin K. A disease often terminating fatally occurs in cattle in the U.S.A. when they are allowed to eat spoiled sweet-clover hay. Autopsies reveal generalized haemorrhage, and it has been found that this ailment is a vitamin K deficiency generated, not by the absence of vitamin K from the ration, but by a toxin present in the spoiled hay (188). This toxin has been identified and synthesized. It is 3-3′-methylene-*bis*-(4-hydroxy coumarin), and is now known by the simplified name dicumarol. Its toxicity is due to the fact that it inhibits the action of vitamin K in the living animal's blood by prolonging the clotting time. It has, however, no anticoagulant effect when added to blood *in vitro*. The anticoagulant activity of dicumarol may be prevented by increasing the cow's intake of vitamin K.

King, Campbell, Rupel, Phillips & Bohstedt (188) showed that dicumarol caused, in growing cattle, an increase in the number of platelets in the blood, but that it had no effect on calcium, fibrin or haemoglobin. Evidence was also obtained in these experiments of the presence in the lipids of alfalfa of some factor, other than vitamin K itself, which is able to reduce prolonged times of clotting of blood.

Sykes & Moore(189) report lesions of the Purkinje network of the bovine heart resulting from K deficiency.

Vitamins of the B group

A few papers have appeared since the last review(190) on the B vitamins in ruminants, and it is now generally agreed that these are not needed in the ruminant's diet owing to their synthesis by ruminal and possibly caecal and colonic symbiotic bacteria and possibly also by yeasts (see previous section on the rumen digestion). Thus Norton(191) found no effect from adding yeast to the rations of lactating cows. There are still, however, many puzzling features about the metabolism of the B vitamins in the ruminant. The first is the fate of the B vitamins which have been shown by Kohler(192) to be present in grass; the second is the conflict of opinion between different investigators as to the synthesis of aneurin in the rumen. Lardinois, Mills, Elvehjem & Hart(83) have shown by microbiological methods that in the presence of urea and readily available carbohydrate the synthesis of riboflavin, nicotinic acid, pantothenic acid and biotin in the rumen was increased, while that of folic acid was uninfluenced. Thiochrome estimations failed to show any synthesis of aneurin, thus confirming the earlier finding of Hunt, Kick, Burroughs, Bethke, Schalk & Gerlaugh(193). The origin of aneurin in milk is still therefore in need of explanation. Possibly the absence of aneurin in rumen contents is due to some source of an antithiamine factor in the rations so far used. That this supposition may be more than a mere hypothesis is suggested by the work of Bhagvat & Devi(194), who found evidence of the presence in cereals, pulses and oil seeds of a substance capable of destroying aneurin. It was not enzymic in nature and was therefore distinct from the Chastek enzyme found in certain tissues of raw fish. An antithiamine substance has also been reported as the probable cause of poisoning of cattle by bracken(195).

Holmes, Lindquist & Greenwood(196) found less riboflavin in the milk of cows and goats at pasture than after stall feeding. Variations of riboflavin from sample to sample were big. Holmes & Holmes(197) had earlier noted only small variations throughout the year of riboflavin in milk (1·13–1·75 p.p.m.), and Johnson, Maynard & Loosli(198) showed that riboflavin output in milk of cows and goats is independent of intake. When goats were fed purified rations evidence was obtained that goats synthesize aneurin and riboflavin. Hunt, Burroughs, Bethke, Schalk & Gerlaugh(199) noted that, when maize was omitted from the diet of a steer with a rumen fistula, riboflavin synthesis ceased. Increase of maize increased the synthesis, which was greatest when the maize was ground. On a partly synthetic ration synthesis tended to increase with carbohydrate content. In these experiments the failure to prove the synthesis of aneurin was again demonstrated.

Theophilus & Stamberg(200) have investigated the effects of diet and other conditions on the riboflavin content of the milk of four cows over a 10-month period. They also found that feeding of sunflower silage increased markedly the riboflavin content of milk, while sweet clover and wheat pasture had no such effect.

In view of the fact that the essential nature of choline in normal animals was first disclosed by experiments with the B-complex, it is of interest to note that excess of this substance in wet brewers' grains was found by Bondi & Meyer(201) to cause interference with reproduction and gestation in cattle.

Lundquist & Phillips(202) administered chlorbutanol through a rumen fistula. They found that the chlorbutanol had no effect on the synthesis of various factors of the B-complex in rumen contents.

Vitamin C (ascorbic acid)

Many studies have proved that the cow, goat and sheep can synthesize ascorbic acid. Thus Vavich, Dutcher & Guerrant(203) maintained a Holstein cow on a diet very poor in C. It gave birth to a normal calf and had vitamin C in blood plasma within the normal range. 150 g. crystalline vitamin C introduced into the rumen through a fistula were rapidly destroyed, but the ascorbic acid in dried grass was more stable. The excretion of vitamin C in the milk alone

was much in excess of that in the food. Urinary excretion of vitamin C was subject to great variations. Wallis(204) had earlier found that in each of three Holstein cows output of vitamin C far exceeded intake, the output in the faeces being greatest, that in the urine smallest, and that in the milk being intermediate. In view of the great faecal output Wallis suggested that tissues of the alimentary tract were responsible for the synthesis of vitamin C. He also found variations of vitamin C in blood and milk due to variations of diet, but bearing no relation to the content of the diet. Rasmussen, Cole, Miller & Thorp(205) obtained evidence from pregnant ewes suggesting that there might be a factor in alfalfa hay and in yeast which stimulated synthesis of ascorbic acid. Holmes, Jones & Wertz(206), however, found no change in the ascorbic acid, aneurin, or riboflavin content of milk of cows of various breeds when they were changed from winter rations to rapidly growing grass.

Bortree, Huffman & Duncan(207) found large increases of vitamin C in the blood plasma of cows, heifers and bulls to which the anaesthetic chlorbutanol had been administered even when the doses were continued for as long as 105 days. Doses insufficient to produce signs of incoordination in the animals (5–10 g./day) caused no ill-effects over long periods, but increased the vitamin C in the blood plasma. This drug stimulates the cow to synthesize ascorbic acid. It may be noted that Phillips, Lardy, Boyer & Werner(208) claimed that injections of ascorbic acid increased the rate of conception in cows which were 'hard to settle', but that they had no effect on cystic ovaries. Barker(209) claimed that this technique was successful with only one of six cows treated. Claims about the effect of ascorbic acid on the reproduction of cows and bulls are still in need of confirmation.

Minerals

For reviews on minerals in animal nutrition the reader is referred to the bulletin by Russell(210), the review by Bohstedt(211) and to the papers by Green(212), Mitchell(213), Martin(214) and Bromehead(215).

Copper. The West Australian investigators have continued their earlier researches on the ailments of cattle and sheep due to deficiency of available copper in soils of certain areas. Teakle & Turton(216) have analysed oats and subterranean clover from various soils for copper, zinc and manganese and found variations by factors of 10 in both copper and manganese contents, as well as deficient zinc contents. Beck has studied the effect of normal and of copper-deficient grazing on the concentration of copper in the milk(217) and the blood(218) of sheep and cattle. Colostrum was not examined, but it was found that there was more copper in milk earlier in lactation than later. Bennetts, Harley & Evans(219) have described the pathology of 'falling disease', the fatal symptom of which seems to be myocardial fibrosis. In areas where 'falling disease' (which Bennetts *et al.* have found to be the fatal termination of acute deficiency of copper) occurs, Beck(218) had earlier found that the concentration of copper in the milk of sheep and cows was reduced. Beck could find no absolute correlation between blood copper and the development of anaemia. The history of the elucidation of the role of copper and cobalt in ruminant nutrition in Western Australia and in South Australia has been described by Martin(214).

Rusoff(220) has reviewed some of the literature on the distribution of copper in the animal body and has analysed many organs of normal and 'salt-sick' calves. In the sick animal he found the copper content of the liver to be normal, of the blood to be above normal and of the bones to be below normal. Krzywanek & Brüggemann, in three papers(221), have made detailed investigation of nitrogen, sulphur and nine other minerals including copper in an animal fed solely on 'Lecksucht' hay. In an area of 160–300 square miles of reclaimed peat land in New Zealand, Cunningham(222) has found that 'peat scours' in cows depastured there is due to deficiency of copper. Administration of copper either to the land or to the animals cures the disease with resulting increase in milk production. The contents of copper in the pastures, and in the blood and the livers of the cows were found to be subnormal.

Molybdenum. The investigations by Ferguson, Lewis & Watson(223) have solved an old mystery as to why certain pastures in Somerset caused 'teart' or excessive scouring in cattle. The cause

was found to be an excess of available molybdenum in the soils of the teart areas to which reference has already been made. Molybdenum in the soil passes into the pasture in toxic concentrations and scours result when cattle are allowed to graze such teart areas. The trouble only occurs where there are 20–100 p.p.m. Mo in the surface soil and where the soil is neutral or alkaline in reaction. Clovers accumulate more molybdenum than grasses. Any manurial treatment such as liming which increases alkalinity or which favours increase of clovers is likely to aggravate the disease or even to produce teartness in land otherwise free. Lewis & Watson (224) have written a review of the teart problem and the steps which led to its solution. Lewis (225) has described the geological aspect of the teart problem which has now been reduced to the more academic question as to why a single relatively narrow stratum of the lower Lias formation should be so rich in molybdenum.

One effect of copper deficiency is to produce scours in cattle such as occur in the Dutch polders. This led Ferguson, Lewis & Watson to try copper sulphate as an antidote to teart. They found that copper sulphate administered to the animals in their food or as a drench cured teart even on the worst pastures. Copper was useless when applied to the soil. Further work has shown that teart pastures are of normal copper content, while the Dutch pastures have no excess of molybdenum and respond to top dressing with copper salts. Teart and the disease found in cattle on the Dutch polders are therefore distinct entities, though administration of copper salts to the animals cures both. In further experiments Ferguson (226) has found that dosing cows with 2 g. copper sulphate per day for from $2\frac{1}{2}$ to 5 months causes increases of blood copper from 0·094 up to 0·163 or 0·235 mg. %, while Dick & Bull (227) have reported experiments on the effect of molybdenum in decreasing the copper in the livers of sheep and cows.

Cobalt. Like copper deficiency, deficiency of cobalt has a world-wide distribution (210), having been found in Western Australia, in South Australia (214), in New Zealand (228), in Britain (229, 213), in Michigan (230), Wisconsin (231) in U.S.A., and in Ontario (232) in Canada. It affects sheep, cattle and goats but not horses (210), and causes unthriftness combined with a low value for the haemoglobin in the blood, restorable to normal by cobalt therapy. Forage in deficient areas contains too little cobalt for adequate nutrition of ruminants (210). Numerous observers, among them Filmer (228), Askew (233), Stanton (234), and Stewart, Mitchell & Stewart (235), have shown that the disease may be prevented by top dressing the pastures either with cobalt salts or with certain cobaltiferous limestones. Askew (236) has shown that cobalt fertilization of the soil can double or treble the cobalt content of the pastures. Hay from deficient and healthy areas was examined by Killham (230). The cobalt content of deficient hay was 0·03–0·06 p.p.m., while that of healthy hay was 0·12 p.p.m. Hurwitz & Beeson (237) have analysed a wide variety of food plants for cobalt. Generally speaking the leaves were the richest in cobalt but variations were large, e.g. lettuce contained 0·3 p.p.m. Co, but addition of 0·2 g. Co sulphate per head of lettuce raised this figure to 5·1 p.p.m. Watson (238) could find no effect on the molybdenum content of pastures when limestone with or without cobalt was used to fertilize them. This result should be compared with the observations of Mitchell, Scott, Stewart & Stewart (239) and of Ferguson *et al.* (223).

Calcium and phosphorus. Evidence of phosphorus deficiency due to seasonal variations over a 4-year period of the composition of forage has been presented from New Mexico by Knox, Benner & Watkins (240), who found that in winter forages were less rich in calcium and phosphorus. They noted that although blood inorganic phosphorus was a good indicator of intake of phosphorus, calcium intake was not reflected by blood calcium. In winter New Mexico cattle did not ingest sufficient phosphorus properly to fulfil requirements for pregnancy or lactation. Cows supplemented with phosphorus gave more and heavier calves. Similar seasonal effects on calcium, phosphorus and magnesium were found in twin calves by Krupski, Almasy, Ulrich & Tobler (241) in Switzerland. Palmer, Gullickson, Boyd, Fitch & Nelson (242) have reported delay of sexual maturity of cows and a modification of the usual signs of oestrus. In spite of the unthrifty appearance of these cattle, which were fed largely on prairie hay, ovulation and conception were normal, as also were the resultant calves. Madsen & Nielsen (243), by feeding a cow on a legume deficient in phosphorus for 2 years 8 months, produced experimental parturient haemoglobinaemia

in it during its third pregnancy. The incidence of this disease, say the authors, increased in 1943 when bonemeal became scarce in the U.S.A. Beeson, Bolin, Hickman & Johnson (244) report experiments to show that beef steers need at least 0·18% phosphorus in their rations. On rations containing 0·11–0·15% phosphorus (equal to 1·04–1·63 g. P/day/100 lb. live weight and Ca/P = 5·7–6·8), steers exhibited low blood phosphorus, pica, and a decrease in the efficiency of utilization of their food. Eight weeks was sufficient time for these signs of deficiency to develop. The deficient diet could be made adequate either by bonemeal or by cottonseed meal. Black, Tash, Jones & Kleberg (245) compared the efficacies of bonemeal and disodium phosphate in supplementing a diet poor in phosphorus. Either supplement improved the calf-crop and reduced the calf-mortality of cattle in Texas. Blood phosphorus was less than 4 mg. in all control animals but in only a few of the treated ones.

The war caused a search for substitutes for bonemeal. The chief hazard in the feeding of rock phosphate is that due to fluorine, of which element such phosphate may contain 3–4%. For sheep and cattle the maximum safe intake is 0·01% F in the ration (246). Ignition of the rock phosphate if properly carried out can reduce its fluorine content to safe limits, but of course adds to its cost. Gullickson & Olson (247) have confirmed the value of the defluorinated rock phosphate for the feeding of dairy cattle.

Outbreaks of industrial fluorosis in cattle and sheep have been described by Meyn & Viehl (248), by Cohrs (249), by Hupka (250) and by Boddie (251), while Majumdar, Ray & Sen (252) have found that fluorine intoxication accounts for a disease prevalent among cattle and buffaloes in Hyderabad, formerly thought to be due to deficiency of phosphorus. Majumdar *et al.* found that calcium salts, including calcium phosphate, combatted to some extent the effect of fluorine. Cohrs found that chronic ingestion of fluorine by calves caused osteoporosis, whereas in older animals exostoses of the jaws and long bones occurred. Meyn & Viehl reported a seven- to tenfold increase in the fluorine content of the bones of fluorized cattle.

Rudakov (253) has found that excess of phosphorus as 10% solution of orthophosphoric acid imposed upon an adequate natural diet was more deleterious to East Friesian cross-bred calves than was excess of calcium as calcium carbonate on the same basal diet. The excess phosphorus produced anorexia and retarded growth. Neither excess interfered with storage of calcium or phosphorus.

Other mineral elements. No attempt will be made in this review to describe the extensive literature on the use of thyroxine in stimulating the lactation of ruminants. In Britain and the U.S.A. the effect of the ingestion of thyroxine produced *in vitro* by the iodination of tyrosine-containing proteins of both animal and vegetable origin has been investigated extensively (254). It may prove to have an application in stimulating milk yields at the end of winter when grass is not yet available, and thus help to increase winter milk supply in times of scarcity. Long-term effects on cows to which iodinated proteins have been fed still need further investigation. Thyroxine injected continuously for 4 weeks into milking cows produces, in addition to the usual increases of milk and fat yields, and decrease of weight, a marked negative balance of nitrogen which, however, becomes markedly positive when the injections are discontinued (255). Of recent years there has been a renewal of interest in antithyroid substances, e.g. thiourea (256), thiouracil (257), aminothiazole (258) and paraxanthine (259). Turner, Reineke & Schultze (256) are testing thiourea to see whether it will be of value in accelerating the rate of fattening of animals produced for meat. Landgrebe & Morgan's (260) observations on the toxicity of thiourea would seem to indicate that, apart from expense, thiouracil would be preferable to thiourea for experiments in fattening of animals. Owen (261) has reported the partition of ingested borate between the urine, faeces and milk of the dairy cow. Most of the borax fed was eliminated quickly via the kidney. The very small amounts found in the milk confirmed the observations of Hove, Elvehjem & Hart (262). Huffman, Conley, Lightfoot & Duncan (263) believe they have evidence of an organic factor in maize and alfalfa which facilitates the attainment of a normal concentration of magnesium in the blood plasma of calves, and Mussill (264) has reported sterility in cattle curable by administration of zinc. Danckwortt & Hoffmann (265) have analysed the livers of ten different species of mammal, including cattle and sheep, for this element.

Moxon, Rhian, Anderson & Olson(266) have found, in pursuance of their earlier work, that arsenic combats the toxic effects of selenium on steers grazing seleniferous land. McConnell(267) has studied the respiratory excretion of ingested selenium.

The mode of absorption and of excretion of metals ingested into animals or given by injection has been discussed by Widdowson & McCance(268). Zinc, they say, is excreted via the pancreatic juice. An interesting fact which they quote is the presence of barium in the choroid of the eyes of cattle. In view of the presence of sulphate in blood this concentration of barium is indeed mysterious. The role of cobalt in intermediary metabolism is still to be elucidated.

Colostrum

Colostrum, besides having a greater ratio of lactalbumin and lactoglobulin to casein, is a much richer source than the mammary secretion which follows it of many substances known to be of great importance to the newborn mammal.

According to Phillips(269) it contains twice as much ascorbic acid as the subsequently secreted milk. That a cow's colostrum is richer than milk in carotene and in vitamin A is again confirmed by recent work(270, 271). This is shown to be true of the milk of the ewe(272) and of human milk(273).

Colostrum, besides having a smaller water content than milk(274), is many times richer in the mineral elements, iron(274) and iodine(275). It contains much more vitamin D than milk(276). Recent work has shown that the colostrum of the ewe is 10 times richer than milk in ascorbic acid(277). Theophilus & Stamberg(200) have found colostrum of Holstein and Jersey cows to be $2\frac{1}{2}$–3 times richer in riboflavin than is milk, and Pearson & Darnell(278) have found the same factor for riboflavin in the cow and a somewhat bigger one (5 times) for the ewe. Pearson & Darnell found $2\frac{1}{2}$ times as much riboflavin in ewe's milk as in cow's colostrum. They also found ewe's milk and colostrum to be richer in both aneurin and nicotinic acid than are cow's milk and colostrum respectively, though no such difference could be detected in the case of pantothenic acid.

Comparing colostrum with milk Pearson & Darnell recorded that with both the cow and the ewe there is about twice as much aneurin (thiamine) in colostrum as there is in milk. Differences between colostrum and milk of both cow and ewe in respect of nicotinic acid they found to be insignificant. In both species the milk was richer in pantothenic acid than the colostrum.

Pearson & Darnell believe that the higher nicotinic acid and riboflavin in the ewe's milk and colostrum compared with those of the cow indicates a species difference. However, no data are quoted as to the diets of their animals, and it seems that just as likely a cause might be found in the different synthetic powers of bacteria in the gut, or perhaps in the greater proportion of greenstuff in the diet of the ewe. Because the colostrum of the cow and of the ewe are rich in vitamin A, and because cow's colostrum is also rich in carotene, it is commonly supposed, especially in view of the smaller neonatal mortality of calves to which colostrum has been fed, that these extra supplies of vitamin A over and above what is found in milk are essential to neonatal health. Efforts have therefore been made to see whether the vitamin A of colostrum can be increased by feeding extra carotene as carrots or extra A as cod-liver oil(271). Such efforts have not been successful.

Underwood & Curnow(272) found that the vitamin A of the milk and colostrum of merino and cross-bred ewes was as shown in the table:

	Vitamin A (i.u./100 ml.)	
	Colostrum	Milk 9 days later
Ewes at pasture	1287	26
Crossbred ewes in dry lot	402	18
Merino ewes in dry lot	272	Trace

These investigators ask whether an abundant supply of A is necessary for the wellbeing of lambs.

The fact that at Cambridge, England, where Moore & Payne(279) found large A reserves in the sheep's liver, lush pasture is available for a much longer period of the year than is the case in Western Australia, where Underwood & Curnow's observations were made, raises the question

as to the magnitude or paucity of the reserves in the livers of Underwood & Curnow's animals. It may well be that for both the cow and the ewe there is an upper but not a lower limit to vitamin A in colostrum and milk.

Baldwin & Longenecker (280) found no difference between the component fatty acids of the colostrum and of the milk of the cow.

Loosli (281) has shown that colostrum causes a rapid increase in the carotene and vitamin A in the blood plasma of the calf. Vitamin A shows a tenfold increase in 2 days.

Moore & Berry (282) report that vitamin A and carotene are many times larger in the blood plasma of colostrum-fed calves than they are in the same calves neonatally, by far the biggest increase occurring in the first day's feeding. Both A and carotene in blood plasma remained low in three calves prevented from suckling. Two of the calves died. Subnormal values were found in calves removed from the dam 12 or 24 hr. after birth. Of forty-two newborn calves the blood plasma contained per 100 ml. 3·5 μg. A and 2·1 μg. carotene.

Sutton & Kaeser (283) found that neonatally, calves had low reserves of A, but that reserves of ascorbic acid were higher than at a later stage. Calves receiving colostrum for 7 days exhibited greater concentrations of A in blood plasma than those receiving colostrum for only 3 days. Vitamin A values were much the same in the 7-day calves as in calves receiving colostrum for 3 days and then 10,000 i.u. A/day for the first 21 days.

Allen (284) has demonstrated the value in calf feeding of colostrum stored in the frozen state and that such material keeps for a whole year. It may be fed to calves till they are 63 days old, thus releasing more milk for human consumption.

Gerasimovitch & Volkovskaya (285) have fed calves on pasteurized colostrum without ill-effects. Phillips (286) reports the control of scouring in calves born of dams on an A-deficient ration, by administration of cod-liver oil. Gullickson & Fitch (287) assigned seventy-two calves at birth alternately to each of two groups. One group received cod-liver oil (25–35 ml.), the other did not. Whole milk was given for 30 days and skimmed milk for 6 months, together with alfalfa *ad libitum* and grain and bran mixture. The cod-liver oil group had a smaller incidence of digestive trouble. The cod-liver oil caused Guernseys and Jerseys, but not Holsteins, to increase in weight somewhat faster, though there was no difference in height at the withers.

Sutton, Kaeser & Soldner (270) have found that as parturition approaches carotene and vitamin A in the blood plasma decline for 3 days. This decline is followed by a somewhat slower rise after parturition. These changes are doubtless associated with the prepartum preparation of colostrum in the mammary glands. In some of these cows the decline in carotene and A in the colostrum was also determined.

The globulin of the colostrum may originate directly from blood globulin as distinct from casein which is only found in milk. The power to make globulin is not present in the newborn calf, but globulin passes in the first few days of nursing from the colostrum to the calf's blood via the alimentary epithelium. There is evidence that such globulins passing from the colostrum to the blood are of importance in the neonatal transfer of passive immunity to certain pathogens in animals, which like the bovine have epitheliochorial placentae. Recent observations show that the secretory power of the mammary epithelium, perhaps due to synthesis, perhaps due to selective permeability, varies with the protein secreted. Thus the human mammary gland according to Witebsky, Anderson & Heide (288) is such that *Rh* antibody may occur in breast milk. However, Liebling & Schmitz (289) found that human colostrum was negligible as a transferrer of passive immunity to diphtheria from mother to baby.

Hale, Walker, Maurer, Baker & Jenkins (290) found no immunity to rinderpest was transferred from pregnant dams, actively immunized by means of an attenuated virus, to the calves to which they later gave birth. No neutralizing antibodies could be found in the colostrum of cows immunized against rinderpest.

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SECTION B. BACTERIOLOGY AND MYCOLOGY APPLIED TO DAIRYING

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More than 5 years have elapsed since the publication of a Biennial Review of Bacteriology and Mycology Applied to Dairying. During this period there has been some diminution in the volume of published papers dealing with this subject, but not to an extent which would allow the information available to be dealt with comprehensively in the space at our disposal. Accordingly, it has been necessary to compress the matter more than is usual and to omit entirely certain sections, notably that dealing with butter. To meet to some extent the convenience of readers, a bibliography showing the main trends of research on butter is included.

I. MILK CONTROL

An important feature of milk control during the period under review has been the introduction, in this country, of the National Milk Testing and Advisory Scheme. Previously official testing of raw milk was confined to designated supplies. It is true that for many years spasmodic tests of other milk supplies had been made by local authorities and by the county agricultural advisory services; schemes for the testing of milk from their own producers were also instituted by the larger dairy companies, but, in the main, control of milk quality and facilities for advising the mass of milk producers were totally inadequate. To avoid wastage through souring which, during the early years of the war (1), threatened to have almost catastrophic proportions, the Scheme, based on the findings of a pilot experiment in four counties during the previous year, was launched in the early summer of 1942. Its objects have been outlined by Kay (1), and details of its administration are given in a publication issued by the Ministry of Agriculture and Fisheries (2). It involves examination by the 10 min. resazurin test of all milk of doubtful keeping quality on its arrival at the dairy platform, fortnightly examination of *all* supplies by a routine resazurin test, and speedy advice on the farm to those producers whose milk is shown to be of inferior keeping quality. Testers and laboratories at commercial dairies operate under licence, and fortnightly visits are made by Ministry of Agriculture bacteriologists for supervision, thus ensuring uniform methods of testing. These bacteriologists test the milk of producer retailers and supplies to dairies not equipped with their own laboratories; it is, in addition, a part of their function to control the efficiency of churn washing and to advise on methods of cleaning and sterilization of churns, plant and milk handling at all dairies. A survey by Thomas (3) of the progress made since the introduction of the Scheme showed that, in spite of the many difficulties which had to be overcome, by the middle of 1945, 384 licensed creamery laboratories and fifty-six Ministry of Agriculture laboratories were making routine fortnightly tests of the milk supplies of approximately 130,000 wholesale producers and 14,000 producer retailers, representing 86% of all milk producers in England and Wales. Similar rapid progress with the routine bacteriological examination of washed churns and with other activities under the scheme was also recorded. A scheme similar in its conception but differing in certain details of operation was launched in Scotland (4) in 1946.

A further feature of no less importance has been the granting of powers—not yet fully implemented—under a Defence Regulation (55G) for the compulsory heat treatment of all milk supplies, other than Accredited or Tuberculin Tested, in urban areas with a population of not less than 10,000. An interesting innovation is the adoption of a methylene-blue test for the bacteriological control of the heat-treated milk together with the phosphatase test for the efficiency of heat treatment (5). With the substitution of these same tests for the plate count in the examination of *Pasteurized* milk (6), colony count methods are no longer used for the control of milk in this country. In Scotland a phosphatase and coliform test has been substituted for the plate count in the examination of *Pasteurized* milk (7).

Colony count methods

Although from the public health point of view incubation of plates at 37° C. may sometimes be desirable, there is no question that for control purposes generally within the dairy industry the use of a lower temperature, such as 32 or 30° C., is preferable. In the eighth edition of the American *Standard Methods for the Examination of Dairy Products* (8) (details of corrections in the text in a reprint of this edition will be found in *Amer. J. publ. Hlth* (1944), **34**, 303) incubation at either 37 or 32° C. is allowed. Incubation at the lower temperature not only leads to higher counts in general but for some purposes may be essential to ensure a true assessment of the bacterial flora. This is particularly the case with milk and milk products that are, during processing, subjected to some form of heat treatment. Abdel-Malek (9) has shown that incubation of plates, prepared from samples of laboratory-pasteurized milk, at 37° C. instead of 30° C., may give a completely false picture of the surviving flora, since heat-resistant corynebacteria (microbacteria) and some micrococci may fail to form colonies on plates incubated at the higher temperature. Mattick, Hiscox & Crossley (10) have also found that with dried milk, colony counts are for the same reason much higher when plates are incubated at 30° C. instead of 37° C. Hempler (11), using a tryptic casein digest lactose milk agar, which, of six media tried, gave the highest counts, found that incubation of plates at 32° C. instead of 37° C. increased the average count of raw and pasteurized milk by 32 and 57% respectively. Counts, using this medium without the addition of 1% milk, were lower for pasteurized milk but were not significantly different with raw milk. He attributed the higher counts which were obtained with tryptic casein digest medium, as compared with a peptic casein digest medium adjusted to the same nitrogen content, to more advanced decomposition by tryptic digestion or more probably to the derivation of growth-activating substances from the pancreatin used for digestion.

A series of papers by Morgan, Anderson & Plastridge (12, 13) and Seeley, Anderson & Plastridge (14, 15, 16) show that although occasionally the presence of *Streptococcus agalactiae*, *Str. uberis* or staphylococcal infections in a herd may materially increase the total count of bulk samples, conditions of handling the milk are in general of greater importance in determining the magnitude of the count. Fuller & Sparkes (17) have studied the effect on the plate count of the method used for shaking before withdrawing portions for plating. When raw market milk and inoculated sterile milk was shaken with or without the addition of glass beads for 2 or 5 min. with rates of shaking of 200, 400 or 900 per min., colony counts were not materially increased over those obtained by standard plating procedures. Several wetting agents tried did not give sufficiently improved results to justify their use for routine purposes.

Gambrell & Ostrolenk (18) were able to reduce the incidence of airborne infection during plating by introducing, in their laboratory, seamless-top tables, dust-proof cabinets and removing obsolete furniture and fittings. The incidence of infected plates poured was reduced from 17·5 to 3% after remodelling. Washing the adjacent wall and plating table with disinfectant just before inoculating and pouring plates did not materially reduce the incidence of infection.

The need for some system of regular supervision of laboratories making official milk analyses is emphasized by the results of a comprehensive survey in forty States in the U.S.A. by Black (19). None of the 281 laboratories surveyed followed the official methods implicitly. Only one laboratory

prepared equipment and media correctly, one used correct methods of plating and incubation, plates were counted correctly by only eleven laboratories and the results recorded and reported correctly by only twenty-five. The inherent errors of the plate count are such that comparable results in different laboratories can only be assured by strict adherence to the detailed techniques laid down. Barkworth, Irwin & Mattick (20) have reported the results of an experiment in which three laboratories tested subsamples prepared from the same bulk and shipped on ice to standardize transit conditions. Each laboratory tested the samples in duplicate on both plain agar and milk agar at 37° C. The results showed that when standard technique is adhered to rigorously, colony counts are comparable between laboratories.

The increasing attention given to thermoduric organisms in raw milk, for the detection of which colony count methods are generally used, has created a new interest in methods that are less time-consuming than standard plating techniques. A loop method has been proposed by Moldavan (21). A calibrated loop projecting from the open tip of a pipette containing 1 ml. of diluent is inserted in the milk to be tested, withdrawn and transferred into the mouth of a sterile tube into which the milk is washed off the loop with the diluent; sterile agar is then added to the tube, which is then incubated either in the upright or sloped position. In a later paper by the same author (22) an apparatus is described suitable for testing 16–400 samples in one operation. No claim is made for absolute accuracy by this method, but rather an estimate which, as the author quite rightly points out, is all that is required for the detection of those supplies likely to give excessively high counts after pasteurization. Rowlands (23) has also found a loop, calibrated to deliver 0·01 ml., suitable for inoculating plates for detecting samples with a high thermoduric content; 1 plate per sample is used, and the milk is removed from the loop by dipping in 1 ml. of Ringer solution previously inoculated into the plate. Moldavan (21) has described a novel method of calibrating 0·001 ml. loops, although no figures are given as to its accuracy; successive loopfuls of milk are rubbed against the aperture of a Breed pipette with its tip uppermost. Prouty, Bendixen & Swenson (24) have described a motor-driven apparatus for the roll-tube method. They used tubes, containing 7 ml. of agar medium, with a constriction 2–3 cm. from the top, and a calibrated loop for the inoculations. Difficulties were experienced in counting, and accumulation of free moisture during incubation encouraged spreaders. In general, counts were lower than by the plate method, especially with high-count milks, but the variances with the two methods were considered about equal. A modification of the Frost little-plate method has been proposed by Bryan & Bortree (25). After making a little plate from 0·1 ml. of a mixture of 1 ml. of milk and 1 ml. of agar held at 50° C., the mixture is laboratory pasteurized before making a second little plate. Results, after incubation for 5 hr., for 1375 samples were closely comparable with those obtained by standard plate counts.

Direct microscopic count

Modifications of the Breed method have been suggested by several workers. Olson & Warren (26) have described a device for marking on a glass slide a circle of 1 sq.cm. area as well as a modification of the mechanical stage of a microscope for counting equally spaced fields across two diameters of the circle. Buck (27) has also described a microscopic field spacer. Mallmann, Bryan & Baten (28) found a smear from a 4 mm. (outside diameter) loop spread over an area 4×8 mm. as accurate as the standard pipette technique. They describe an acid stain—1 g. methylene blue in 500 ml. 95% ethyl alcohol acidified with 0·5–5 ml. conc. HCl—which gives a less intensely coloured background than the usual staining methods, and advocate the use of an orange filter in counting. North (29) obtained higher counts with dried milk when aniline oil-methylene blue was used instead of Breed's stain (the American Standard methods (8) erroneously state that the solutions should be made up to a total volume of 55·5 ml. instead of 100 ml.). According to Levowitz (30), counting a strip of smear, from one end to the other with a 0·20 mm. diameter field, is better and simpler than the usual method, and gives reproducible results. When the number of clumps in a strip exceeds 100, 1 strip is counted; 50–100 clumps, 2 strips; 20–50 clumps, 3 strips; and, less than 10 clumps, 5 strips.

Stark & Campbell (31) have reviewed the literature on the direct microscopic method for the examination of pasteurized milk. In their experience living and dead bacteria could not always be distinguished, but the method was useful for detecting the presence of thermophiles. Mallmann & Bryan (32) found that the microscopic method was not entirely reliable for the detection of thermoduric organisms in laboratory-pasteurized samples. The counts in some cases were so much in excess of those obtained by plating methods that it was obvious that dead bacteria were being counted. They found, however, that by holding milk samples at pasteurization temperature for 2 hr. counts by the direct microscopic method agreed closely with those obtained by plating. They also proposed a further method for the detection of thermoduric organisms. Duplicate smears of the raw milk are prepared, one of which is used for the raw-milk count, and the other, after autoclaving for 10 min. at 15 lb. pressure, for the thermoduric count; autoclaved slide counts in excess of 40,000 ml. indicate excessive numbers of thermoduric organisms. Mickle & Borman (33), Gunderson & Anderson (34), and Anderson (35) favour the use of the Breed microscopic count for the examination of pasteurized milk, because of its rapidity, its qualitative as well as quantitative character, and especially because of its value in detecting the presence of thermophilic and thermoduric types which may be overlooked when agar plates are incubated at one temperature only.

Dye reduction tests

The effect of inversion on the methylene-blue reduction time continues to be investigated. Seshacharyulu & Kothavalla (36) found that the reduction times for both raw and pasteurized milk samples were the same whether the tubes were inverted at $\frac{1}{2}$, 1, $1\frac{1}{2}$ or 2 hr. intervals, but longer intervals increased the reduction time. It has again been shown (11) that when tubes are inverted every $\frac{1}{2}$ hr., duplicates agree better, reduction times are shorter and the results are better correlated with colony counts—r is $-0{\cdot}84$ as against $-0{\cdot}70$ when tubes were not inverted. Barkworth *et al.* (20) found that the reduction time for a given plate count (37° C.) was affected by the season of the year, the meal of milking and the age of testing. Production conditions, as judged by a score card designed to give the greatest emphasis to those factors known to be of the greatest importance in influencing milk contamination, were not particularly well related either to the methylene-blue or to the colony-count results. According to Johns (37) the methylene-blue test is a better measure of utensil contamination than the plate count, since bacteria derived from unsterile utensils tend to occur in clumps and colony counts are in consequence much lower than is actually shown to be the case by direct microscopic counts. The methylene-blue results for 95,000 milk samples tested in Australia have been analysed by Smythe (38). The proportion failing a 4 hr. standard followed the mean atmospheric temperature curve fairly closely; approximately 10% of samples failed when the mean temperature was less than 50° F., but when the mean temperature exceeded 75° F. more than 50% failed. He concludes that the results are affected more by temperatures at which milk is stored before testing than by conditions of production. Experiments in which samples of morning milk, received in the laboratory within $2\frac{1}{4}$ hr. of completion of milking, were divided into subsamples which were stored for 5 hr. at different temperatures ranging from 20 to 35° C. showed that the methylene-blue reduction time decreased as the temperature of storage increased. Storage for 5 hr. at 20, 25, 30 and 35° C. respectively gave a mean shortening of the reduction time of approximately 1, 2, $3\frac{1}{4}$ and 4 hr.; at 15° C. the reduction time remained almost unchanged, but below this temperature there was a slight increase in reduction time during storage; the reduction times initially ranged from $3\frac{1}{2}$ to $7\frac{1}{4}$ hr. Any bactericidal property possessed by the milk was without effect on the methylene-blue reduction time of samples stored at 20° C. or higher. There was no correlation between the initial quality and the time for reduction, but relative deterioration was greater for milks with low initial colony counts. He regards the testing of samples at the time they arrive at dairies as unsatisfactory for grading purposes, since it may give an unfair advantage to those producers whose milk arrives early.

Thomé(39) has studied the change in flora during the incubation of samples in the methylene-blue test. The proportion of the different types, which consisted mainly of streptococci, micrococci and coliform organisms, changed during incubation. Initially the flora of different samples consisted of 7–100% streptococci, 0–95% micrococci and 0–6% coliform organisms; at the time of reduction the proportions were respectively 20–88, 9–65 and 0–24%. The reducing ability of pure cultures of representatives of the three groups, in terms of the time taken for an inoculum of 1 million organisms per ml. to reduce methylene blue in 'sterile' milk, was approximately the same, though at the time of reduction counts were higher for streptococci and coliform organisms than for micrococci. With mixed cultures, the rates of increase of each type were changed with varying proportions of inoculum, although the time for complete reduction was not materially affected. Morris(40) has observed that proliferation of psychrophilic bacteria of the genus *Pseudomonas*, derived from the water supplies used on the producing farms, may lead to a shortening of the reduction time after overnight storage of milk samples in a refrigerator.

Many papers dealing with different aspects of the resazurin test have appeared during the period under review. Resazurin has been synthesized(41) and standards have been suggested for preparations intended for milk testing; these standards have been adopted for resazurin used for official milk tests in this country(42). According to Johns(43) resazurin tablets approved for use in North America are approximately 60% stronger than those used in this country. The use of the stronger solution made colour differentiation easier, especially when dealing with cream, colostrum, and milks tending to give a grey tinge when mixed with the dye, but it delayed by about 30 min. the time taken to reach the pink stage, although there was no difference in the time taken to reach the Munsell P7/4 colour standard (about half-way between the initial blue and the full pink stage). Thomas(44), however, found that doubling the concentration of resazurin only slightly delayed the time taken to reach Lovibond disk 4, 2 or 0.

The interpretation of results obtained by workers in different countries is complicated by the use of different colour standards for the intermediate stages of reduction. Golding & Jorgensen(45) have compared the colour standards of a Munsell colour grader with those of the Lovibond disk; the average disk values were 6 for Munsell A, 4·95 for B, 3·85 for C and 2·35 for D. In some European countries a colour chart (Dr Kloz) graduated in four classes is used(11): class I corresponds to the initial colour (blue) of resazurin in milk, class IV to the mixture of resorufin and milk (pink), and classes II and III represent the transitions between these two extremes. A method based on a combination of pyrex glass suspensions and various coloured inorganic salt solutions has been developed by Hallinan(46) for the preparation of permanent colour standards for use in the resazurin test.

Davis & Newland(47) found that the down-grading of milks when using the Lovibond comparator in artificial light could be obviated by using either low-pressure mercury-vapour fluorescent lighting, or a specially designed lamp unit for attachment to the comparator, incorporating a glass filter filled with the blue and pink solutions as recommended for use in colorimetry by the Conseil Internationale de l'Eclairage. Thomas(48) found a slightly better agreement with north light when using fluorescent lighting than when using the C.I.E. lamp fitment. Barkworth & Jones(49) and Thomas(48) have shown that some workers fail to follow the prescribed technique(42) which requires that colour readings falling between two disk numbers should be recorded as the $\frac{1}{2}$ value, although errors arising rarely exceed $\frac{1}{2}$ a disk and are therefore unlikely to invalidate the results. Davis, Jones, Newland & Wilby(50) found that the results of the test were not materially affected by a variation in water-bath temperature of several degrees above or below 37·5° C.

It is well known that in milk at 37° C. complete reduction of resazurin and methylene blue occurs in approximately the same time. There is, therefore, no advantage in using resazurin unless it can be shown that the intermediate stages of reduction, which are reached earlier and therefore make the test more expeditious, can be used for grading purposes. The triple-hour test(51) in which samples are classified according to whether they reach a particular stage of reduction in 1, 2 or 3 hr., and the routine test(42) as used in this country, in which the stage of reduction reached in a specific time, are examples of ways in which the test has been used for

grading purposes. It is inevitable, however, that the grading of individual samples by either of these tests may fail to agree with those of the methylene blue or the time taken for complete reduction of resazurin, since it is known that samples reaching the same stage of partial reduction of resazurin in the same time may differ appreciably in the time taken for complete reduction and vice versa. It has been observed (52) that when the Lovibond disk number of a sample is plotted against the time of reading for successive observations on the same sample, the shape of the resulting curve varies widely from one sample to another; although the shape of this time/disk curve was influenced by the number of cells and the number of bacteria present in the milk, these were apparently not the only factors responsible. The effect of cells on the resazurin and methylene-blue test has also been investigated by Davis, Newland & Knuckey (53), Morgan & McKenzie (54), Thomas & Bowie (55, 56) and Morris & Edwards (57). The reducing activity of udder cells was found to be greater in fresh milk than in milk which had been aged for 24 hr. Some milk samples containing about 500,000 udder cells per ml.—a figure which is not unusually high for herd bulk milk (55)—reduced resazurin to disk 4 or even 3 when the incubation period at 37° C. was 1½–2 hr., but a very much larger number of cells was necessary to effect partial reduction with shorter incubation times, and excessive numbers were necessary to affect the time of complete reduction of resazurin or methylene blue. The possibility of differentiating between reduction by cells and bacteria in the resazurin test has been explored by Davis & Jones (58), but of several chemical inhibitors tried none was sufficiently specific in its action, against either cells or bacteria, to be effective.

With the introduction of the National Milk Testing and Advisory Scheme in 1942, the 10 min. resazurin test (59) was adopted for the testing of milk suspected of being unsuitable for acceptance on its arrival at the creamery. Depending on the subsequent disposal of the milk, creameries reject milk giving either a disk reading of 3½ or less or of ½ and 0 only. Barkworth, Egdell, Davis, Rowlands & Watson (60, 61), Thomas & Phillips (62) and Thomas, Phillips & Andrews (63) have compared the results of this test with those of others—½ hr. methylene blue, acidity, 68% alcohol, clot-on-boiling, smell and taste—commonly used for the same purpose. As would be expected they failed to find an exact agreement between the results of the various tests. The majority of samples reducing resazurin to disk 3½ or less in 10 min. reduced methylene blue in ½ hr. or less, had a titratable acidity value of 0·18% or higher, and had a colony count in excess of 30 millions; most samples which clotted on boiling reduced resazurin to disk 0, and those giving a positive result with 68% alcohol reduced to disk 3½ or less. Although a proportion of tainted samples gave disk readings of 4 or higher, many samples, which on a smell and taste basis alone would be considered as suitable for acceptance, were found to be unsatisfactory by the 10 min. test. Wood & McKenzie (64) found that at dairies which confined testing to confirmation of those churns suspected on a smell or taste basis, the proportion of milk rejected (0·13–0·17%) was much less than at a dairy which tested every churn (1·06%). Anderson & Wilson (65) regard a standard of disk ½ or 0 by the 10 min. test as far too lenient for rejection purposes; only 59% of samples reducing methylene blue in ½ hr. were rejected on this basis and 86% when the standard was set at disk 3½ or less.

In the routine resazurin test (42) which is used for the fortnightly testing of producers' samples under the National Milk Testing and Advisory Scheme, the samples, which are taken at the time the milk arrives at creameries or during its retail distribution, are thereafter stored at shade atmospheric temperature until approximately 24 hr. old from the time of production. In an attempt to eliminate the effect of variations in treatment arising out of differences in atmospheric temperature during the pretesting storage period, the time of incubation at 37·5° C. in the resazurin test is varied according to the following temperature compensation scale:

Mean of maximum and minimum shade temperatures	Period of incubation at 37·5 ± 0·5° C. (min.)
40° F. and under	120
Over 40° F. and up to and including 50° F.	90
Over 50° F. and up to and including 55° F.	60
Over 55° F. and up to and including 60° F.	30
Over 60° F.	15

Samples, after incubation for the prescribed times, with disk readings of 4 to 6, 1 to 3½, and ½ or 0, are placed in categories A, B and C respectively. Phillips & Soulides (66) found that the test was highly reproducible and obtained a maximum variation in reading of ½ a disk for one worker and 1 disk for four workers. An analysis (48) of the results for over 400,000 samples has shown that the proportion placed in category C ranged from 2% when the mean temperature was 40° F. or less to 17% when it exceeded 60° F. and 26% when it exceeded 65° F. Modifications of the temperature-compensation scale have been suggested by Thomas (48) and Davis & Ward (67) with the object of reducing the variation in the proportion of category C samples, but it is difficult to see how adequate discrimination can be achieved by using either an incubation time shorter than 15 min. in hot weather or longer than 2 hr. in cold weather.

Results obtained by Thomas, Bowie, Peregrine & Phillips (68) and Anderson & Wilson (65) show that neither the methylene-blue test nor the various modifications of the resazurin test are particularly suitable for measuring the keeping quality of milk. The former authors, using taste and clot-on-boiling as end-points in the keeping quality test at 18° C. with observations at 4 hr. intervals, obtained mean r values of 0·76 for the routine resazurin test and 0·74 for the methylene-blue test. Anderson & Wilson, using 68% alcohol, which was found to be a reliable measure of the end-point of keeping quality, obtained mean r values of 0·70 for the methylene-blue test, 0·69 for the time taken to reach disk 1 and 0·59 for the 1 hr. test. There was a wide scatter of keeping quality for specified standards by either of the dye tests. Both sets of workers obtained slightly higher r values in summer than in winter. Hempler (11) found the 1 hr. resazurin test unsuitable for grading purposes when dealing with samples with colony counts, at 30° C., of less than 1 million; the majority of samples had a tendency, irrespective of the count, to give colour values corresponding approximately to Lovibond disk 4 to 4½. Golding & Jorgensen (45) found that the mean colony count at 37° C. ranged from 124,000 for samples giving disk 6 by the 1 hr. test to over 6 millions for disk 0. Colony counts of samples with the same disk number were, however, widely scattered and overlapped those for samples with widely differing disk values.

The retention of the methylene-blue test for designated milk and the introduction of the routine resazurin test has led to some confusion in the minds of producers whose milk is tested by both methods. Comparisons between the two tests have been made by Egdell & Thomas (69) and Thomas & Peregrine (70). Over 90% of samples satisfying the methylene-blue standard (4½ hr. in summer; 5½ hr. in winter) were placed in category A by the routine resazurin test, but over a third of the samples failing the methylene-blue standard were also placed in category A.

With the possible exception of their use for the detection of thermophiles, dye reduction tests have not found much favour for the testing of pasteurized milk. Recently, however, the methylene-blue test has been adopted officially for testing heat-treated (5) and pasteurized (6) milk in England and Wales and heat-treated milk in Scotland (71). In England and Wales samples, taken during distribution and stored at atmospheric shade temperature (not exceeding 65° F.) until between 9 and 10 a.m. on the following morning before testing by the methylene-blue test at 37° C., must not reduce the dye within ½ hr. In Scotland the dye is added to 10 ml. portions of the samples on their arrival in the laboratory on the day of distribution; the tubes are then stored at atmospheric shade temperature and must still be blue when examined at 12 noon on the following day. The tests, in their present form, are intended to provide an assurance that, under the conditions of distribution and storage by the consumer normally prevailing in this country, milk complying with the standards will have a keeping quality that will satisfy consumer requirements.

In the past, most investigators have applied the dye tests to samples of freshly pasteurized milk, and have found that under these conditions they fail to differentiate between samples with widely differing bacteriological quality. These findings have been confirmed by Rowlands & Provan (72), but they found that if samples were stored at 15·5° C. for 24 hr. before testing by methylene blue or resazurin at 37° C. some indication was obtained of post-pasteurization contamination as measured by the coliform test and by a methylene-blue test at 15·5° C. for keeping quality. In a later paper (73) the same authors proposed a modification of the resazurin test for control of processing operations. Samples of pasteurized milk taken at various points in the

plant were stored at 18° C. for 24 hr. and then tested by the resazurin test at 37° C. with observations at 10 min., ½ hr. and thereafter at ½ hr. intervals for 3 hr. By this test, holder and other uncontaminated samples gave a disk reading of 4 or higher after 3 hr., whereas contaminated samples gave lower disk values; complete reduction in 10 min. or ½ hr. was common with heavily contaminated samples.

The same authors (74, 75) have also reported the results of tests on fortnightly samples of bottled pasteurized milk as distributed by twenty-seven dairies during a 12-month period. They found that, in winter particularly, only a small proportion of samples with a keeping quality at 18° C. of 1 day or less or showing the presence of coliform organisms in 0·01 ml. when tested on the day of distribution were detected by the officially prescribed ½ hr. methylene-blue test (5). Instead of storage at atmospheric temperature before applying the ½ hr. methylene-blue test they advocated storage at 18° C. Complete reduction of resazurin after incubation for ½ hr. at 37° C. gave results very similar to those of the ½ hr. methylene-blue test. Storage at 18° C. was preferred on the grounds that it would give a greater margin of safety to the consumer and would result in more uniform treatment of dairies at all seasons of the year. In a survey of the results of routine bacteriological tests of pasteurized milk McKenzie & Booth (76) found that seasonal variations in the proportion of samples failing the ½ hr. methylene-blue test were greater than with the old colony count (37° C.) standard of 100,000 per ml. The ½ hr. methylene-blue results after storage at 18° C. agreed fairly closely with those after storage at atmospheric temperature, but no samples were tested during the colder months of the year. Their samples were taken on the day of processing and tests were set up within 3 hr. of sampling; such samples may fail to give a true reflexion of the quality of milk as distributed to the consumer, for under the conditions of commercial overnight cold storage, temperatures may not be low enough to suppress bacterial growth completely. Eyrard & Rey (77) have reported the results of tests, made 18–24 hr. after processing, on 2403 samples of pasteurized milk from 116 depots during April–June 1942. They found that 92% of samples reducing methylene blue (no ½ hr. inversions) in 1 hr. had colony counts in excess of 3 millions, whereas 77% of those with counts under 100,000 took over 6 hr. to reduce; between 1 and 6 hr. there was very little relationship between the colony count and the reduction time. They concluded that the methylene-blue test was only of value at the two extremes. Bird & Egdell (78) found that the presence of excessive numbers of thermophiles, as shown by colony counts at 63° C., in samples of pasteurized milk taken from the holder at one plant, caused rapid reduction of methylene blue at 37° C. In this instance the methylene-blue test detected a fault which had previously escaped attention, for owing to failure of the thermophiles to form colonies at 37° C., counts at this temperature were quite satisfactory.

Methods for the detection of coliform organisms

The search for a medium that will encourage the maximum growth of coliform organisms while at the same time suppressing types likely to give false-positive reactions still continues. Mallmann & Darby (79), experimenting with the alkyl sulphates as selective agents, found that sodium lauryl sulphate at a concentration of 1:10,000 in tryptose-lactose broth was the most suitable for the presumptive coliform test. At this concentration it had no toxic effect on pure cultures of *Bact. coli*, and with water it yielded a higher number of positive results with a fewer number of false-positive reactions than standard lactose broth. Hajna & Perry (80) have also reported favourably on this medium for water analysis, and Hupp (81) used this substance at the same concentration in broth to eliminate troublesome aerobic, spore-forming, lactose-fermenting bacteria. In experiments on the use of this medium for testing milk samples using inocula of 2, 5 and 10 ml., Gardner, Garrett & Prickett (82) obtained the best results with an inoculum of 5 ml. and a concentration of 1:5000 of sodium lauryl sulphate. They experienced some difficulty in reading the results owing to the tendency for the milk to curdle during incubation causing blockage of the inverted gas tube. The medium was just as sensitive for the detection of coliform organisms in milk as brilliant green bile lactose broth, although the latter medium had a tendency to fewer

false-positives. Leifson (83) found that of six bile salts tested, sodium hyodesoxycholate at a concentration of 0·01% in lactose-peptone broth was the most suitable for the estimation of coliform organisms in milk and water. Coliform colonies on agar, prepared from this medium and containing 1:50,000 of neutral red, were similar in appearance to those on desoxycholate agar. In the United States (8) two media—brilliant green lactose peptone bile and formate ricinoleate broth—are suggested for the detection of coliform organisms. Using the dilution extinction method and inoculating ten replicate tubes from each dilution of milk into each medium, Skinner, Marvin & Honaas (84) found no significant difference between the number of positive results with the two media. They ascribed the more frequent occurrence of false-positive results with the formate ricinoleate broth to the formation of gas from the formate by *Proteus* types, and considered this to be an advantage when in addition to the coliform organisms an estimate of *Proteus* and *Salmonella* types in water or milk was required.

It has been shown by Barkworth (85) and Barkworth & Irwin (86) that the presumptive coliform test underestimates the population in water and more so in milk. Using a non-clumping strain of *Bact. coli*, they inoculated water and aseptically drawn milk to give in each a final concentration of 0·5 organisms per ml.; thirty tubes of McConkey broth were then inoculated with 1 ml. quantities of the milk and thirty with 1 ml. quantities of the water. The proportion of positive tubes was 27% with water, and 23 and 19% with milk stored for 24 hr. before inoculation at 40 and 72° F. respectively. The results were unaffected by the presence of bile salt which, at the concentration used in the test, was shown to have no bactericidal effect. The same authors (87) found that although when testing small volumes in $6 \times \frac{5}{8}$ in. test-tubes there was no significant difference in the number of positives with rimmed or rimless $1 \times \frac{3}{8}$ or $1 \times \frac{5}{16}$ in. gas tubes, the use of too small a gas tube when using a large inoculum (50 ml.) led to a number of false-negatives. No significant difference in the number of positive results was observed with 50 ml. inoculations of water into either 8 oz. kali-bottles or $7 \times 1\frac{1}{2}$ in. test-tubes using $3 \times 1\frac{1}{2}$ or $4 \times \frac{3}{4}$ in. gas tubes. Katzin, Strong, MacQuibben & Itzkowitz (88) found that the use of 0·85% salt for preparing dilutions may lead to loss of ability to produce gas in lactose broth and to false-negative results in the presumptive coliform test. Archambault & McCrady (89) have observed that liquid test media which have been stored for long periods at low temperatures may contain sufficient dissolved air to produce visible bubbles of gas in Durham's tubes when incubated at 37° C. These bubbles appeared in tubes which had been stored for 7 days at 4° C. or even at 13° C. To avoid errors they advocate storage of tubed media at 25° C.; where this is impossible tubes of media should be incubated overnight at 37° C. before inoculation, and those showing bubbles discarded.

Morris (90) has encountered strains of intermediate coliform types which were highly sensitive to a bactericidal substance and a growth-inhibitory factor present in raw milk. They were isolated from samples of designated milk which, in spite of giving a positive presumptive test in the 0·001 ml. dilution, reduced methylene blue and resazurin very slowly. In a later paper (91) it was shown that the organisms grew normally in raw milk which had been heated to 53° C. for 30 min., but were inhibited in the same milk heated to 52° C. for the same time. Malcolm (92) has studied the effect of temperature on the growth of coliform organisms in milk in an attempt to explain the results of previous work in which it was found that in raw milk in Scotland, *aerogenes-cloaceae* strains predominated in summer and *Bact. coli* in winter. When grown in milk either in pure or in mixed cultures with *Bact. coli* for 24 hr. at 17 or 22° C., *Bact. aerogenes*, *Bact. cloaceae* and *Bact. oxytocus* multiplied more rapidly than *Bact. coli*; at 30 or 37° C. the reverse was the case. When fresh bovine faeces were inoculated into raw milk and incubated at 17° C. for 36 hr., enrichment of *aerogenes-cloaceae* strains occurred, and in some cases they formed the predominant coliform flora. In summer, therefore, when conditions for multiplication are favourable, these types may predominate in milk though the contamination may be of faecal origin. Robinton & Genung (93) found that in sterile 30% cream inoculated with pure cultures of *Bact. coli* and *Bact. aerogenes* fairly rapid multiplication occurred during storage at 8° C., a finding which is somewhat surprising, bearing in mind the known slow rate or proliferation of these organisms in milk stored at 10° C. or lower.

Although coliform organisms are known to cause undesirable fermentation leading to loss of keeping quality in milk, they have seldom been implicated as the specific cause of taints. Claydon (94), however, attributed the development of a pronounced medicinal flavour in 1–3 days in pasteurized milk to the action of two closely related strains of *Bact. aerogenes*. The organisms were destroyed by pasteurization at 143° F. for 30 min., and the infection was traced to water in the tanks in which the bottles were washed. McKenzie, Morgan & Parker (95) have emphasized the importance of careful confirmation when *Bact. aerogenes* is suspected to be the cause of ropiness in milk. In a case they investigated it was found that micrococci which originated in hay dust were responsible; *Bact. aerogenes* was at first suspected. Colonies on agar which at 48 hr. were apparently pure cultures of the latter organism were later found to contain the micrococci, and the greatest difficulty was experienced in separating the organisms.

Methods for assessing the sterility of milk equipment

In arriving at a measure of the efficiency with which milk equipment has been cleaned and sterilized it is possible to approach the problem from two different angles, according to whether a measure is desired of the probable effect of the residual flora on the bacteriological quality of the milk or of the efficiency of the apparatus and methods used. The former object can be achieved by suitable bacteriological tests on samples of the same bulk of milk taken at various stages in production or processing, and results obtained in this way are invaluable for tracing sources of contamination likely to affect the commercial life of the milk. A better assessment of the efficiency of the apparatus and methods used for cleaning and sterilization is possible by estimating the bacteria removed from equipment surfaces by sterile rinses or by swabbing measured areas of surfaces which are not amenable to rinsing. Only a proportion of the bacteria on surfaces are removed by these methods, and careful standardization of procedures used is essential to obtain reproducible results. In this country, techniques for rinsing churns (96, 97) and for swabbing equipment (98) have been issued for advisory tests under the National Milk Testing and Advisory Scheme. For rinsing churns, 500 ml. of sterile $\frac{1}{4}$-strength Ringer solution is used, and the churns are twice rolled so as to make 12 complete revolutions, with an interval of 5 min. between the two rollings. Rolling instead of shaking has been adopted, since shaking causes greater disintegration of clumps and, without a mechanical shaker, standardization of treatment is difficult. Churns, tested within 1 hr. of washing, with a total colony count at 37° C. of not more than 50,000, are classed satisfactory, those with counts between 50,000 and 250,000 fairly satisfactory, and greater than 250,000 unsatisfactory. The swabs are prepared by wrapping a 6 in. length of 2 in. wide unmedicated gauze around the end of a 12 in. stiff stainless steel wire, and sterilized in 25 ml. $\frac{1}{4}$-strength Ringer solution in a 10×1 in. test-tube. The measured area to be tested is covered twice with the same swab, heavy pressure being applied. A colony count per sq.ft. of 5000 or less is classed satisfactory, 5000–25,000 fairly satisfactory and greater than 25,000 unsatisfactory. Experiments (99) made before the technique was adopted showed that approximately 50% of the total organisms were recoverable from a surface; light pressure removed about half as many organisms as intermediate or heavy pressure, which agreed closely; only about 20% of the total organisms were retained by the swab itself; and the swabbing of adjacent areas gave reasonably reproducible results. Results obtained by this method when applied to dairy, meat-canning and bakery plant have been reported by Crossley (100). As would be expected the higher counts obtained by incubation of plates at 32° C. instead of 37° C. increased the proportion of unsatisfactory results. Colony counts at either temperature were in general much higher with equipment, such as road tankers and large storage tanks, which is not easily sterilized by heat, than with plant which is readily dismantled for cleaning and sterilization. Cotton swabs are usually advocated by American workers (8, 101, 102). These methods involve elaborate procedures for disintegrating the swabs before plating to obviate an underestimate of the organisms removed from surfaces due to retention of bacteria in the swabs.

A modified rinse technique for testing the sterility of washed milk bottles has been proposed

by Hobbs & Wilson (103). Instead of the usual method of shaking, they advocate gentle rolling of bottles to wet the whole surface, the process being repeated after an interval of 15–30 min. Colony counts by this method were slightly higher and more reproducible than by the shaking method.

The merits of paper-board milk cartons which, apart from the question of cost, meet most of the requirements of a satisfactory container for distribution of fluid milk, have been well recognized for many years. A warning against too rigorous bacteriological standards for paper used in the manufacture of cartons has been given by Ball (104) on the grounds that the number of bacteria present has little public health significance. He has also emphasized the importance of adequate controls in laboratory tests; parallel tests on sterile flasks indicated that laboratory contamination was largely responsible for positive results with waxed containers. Foord, Crane & Clark (105) found that when corrections based upon the use of adequate controls were made in sterility tests of carbons, the proportion sterile was increased from 79 to 98·6%. Hoy (106) found that 97% of cartons examined during a period of 6 years contained less than 200 recoverable bacteria per carton and 86% were virtually sterile.

II. CHEESE

The exigency of war lent an impulse to some lines of cheese research, especially in U.S.A., Canada, New Zealand and Australia. The demands made on the cheese-making industries of the supplying countries by the armed forces and by Lease-Lend programmes required increased production with a minimum of low-grade samples. Methods were sought for increasing the rate of ripening of cheese, so lessening the essential time between manufacture and consumption. At the same time it was necessary that the cheese should be of acceptable quality and free from serious defects. Again, suitable materials, other than tin which was scarce, were needed for packing cheese and other dairy products for transport and storage. Thus, although cheese research has not been entirely directed towards these immediately utilitarian ends, it has been in abeyance in some countries, and the subject-matter of this section has been drawn from more restricted sources than usual.

It is a well-established fact that pasteurization of the cheese milk eliminates many of the common faults of cheese which are due primarily to micro-organisms introduced with the raw milk. Researches at the New York Agricultural Experiment Station (1) and the University of Wisconsin (2) reiterated the fact that cheese made from pasteurized milk were of a more uniform quality than those made from raw milk, had a smooth texture, were free from gassiness, but milder in flavour, though this seemed to satisfy the taste of a growing number of consumers. To ensure these good points, however, the milk must be of good quality before pasteurization.

Bacteriophage

The increasing use of pasteurized milk for cheese-making has created special problems. One of the most serious of these is the incidence of 'slow working' in the cheese vats due to the presence of bacteriophage active for the lactic acid bacteria used as starters. This was appreciated by Whitehead and his colleagues in New Zealand as long ago as 1936, and the trouble has since been met and studied in most countries where Cheddar and other hard-pressed cheese are made on a factory scale. Reports of recent researches have come from New Zealand (3), Australia (4), Canada (5), France (6), Russia (7) and England (8). English workers (9, 10), following more or less closely the pioneer work of Whitehead and his co-workers (see Biennial Reviews for 1939 and 1941) (11), show that the danger of slow working and even of complete 'pack-up' is not entirely eliminated by using mixed starters instead of the single strains used by New Zealand cheese-makers. Anderson & Meanwell (9) consider that it is wiser to rely on multiple, rather than on single strains until means have been found to prevent sudden failure of the latter. Johns (5) also reports outbreaks of slowness (but not 'pack-up') from two creameries using commercial mixed

starters. Nichols & Wolf(10) examined starters from various English dairy schools and cheese factories during 1942. Bacteriophage was widely distributed and appeared to be prevalent in some factories, while it was not found in others. Phage attack was widespread and could not be correlated with heat treatment of the milk. From the study of the phage-organism relationships it was possible to deduce the relationships of the starter strains and their phage races, and in some cases to identify the starter strains circulating in commerce. The 'nascent phage' phenomenon was not common among fourteen selected strains and their corresponding phage races. It is possible that the reaction may indicate fundamental relationships which may serve as further aids to classification of strains and phages. The potential danger of this phenomenon in the management of starters had already been pointed out by Anderson and Meanwell(9) and Hunter(12).

The true origin of bacteriophages is still obscure. Jensen(4) has observed that phage may be found in filtrates from the milk cultures of certain strains of starter organisms to which rennet had been added at the time of inoculation. Further experiments have strengthened his view that some strains of lactic streptococci may act as 'carriers' of phage which is released when the organism grows in milk containing rennet. Whitehead & Hunter(13) have modified their views on the origin of bacteriophage within the culture itself. They point out that most of their observations could be explained on the basis of contamination with airborne phage infection which may be heavy in the neighbourhood of a commercial dairy, and there seems no doubt that this is the most important immediate source. The surroundings of cheese factories may be heavily infected with phage particles disseminated in the air with dust and droplets of whey. Whey from infected vats is perhaps the most frequent source of this phage, but there are serious implications in the findings of Nichols & Wolf(14), who demonstrated the presence of phage in bacteria-free filtrates of cheese after storage for a year. There is danger of reintroduction of infection from cheese held over from a previous season. The proximity of piggeries established to consume whey from the factories may constitute another source of phage infection. Mazé(15) demonstrated, in the excreta of a pig from such a piggery, five different phages specific for five of the starters used in the factory. He did not find phage in the factories when there were no attached piggeries and the whey was transported to a distance. Nichols(16) instances certain creameries which had experienced neither phage infection of the whey nor slow working in the cheese throughout three consecutive seasons, although no special precautions had been taken to protect the starter from whey contamination. She suggests that the methods of whey disposal at a factory may affect the incidence of phage attack.

Whitehead & Hunter(13) pointed out that the seriousness of the risk of airborne contamination in the laboratory is not generally appreciated. (In this connexion photographs by Johansson & Ferris(17) illustrating the scattering of droplets by the blowing out of a pipette during plating operations are illuminating.) In their experience the occurrence of phage in the air is spasmodic, depending on various factors, but at times even the transfer of stock cultures without contamination is difficult. The danger of contamination of bulk cultures is even greater. Cheese vats expose a large surface to airborne infection, and as Hunter(18) has shown there may be, in addition, a cumulative infection from all parts of the plant and from milk cans which have been used to carry whey from the cheese factory to the farm. The normal daily routine of cleaning should, however, generally reduce contamination sufficiently to avoid trouble during the 6–7 hr. in which the starter organisms are required to continue producing acid in the vats. Whitehead & Hunter(3) have suggested that the whey might be pasteurized as it runs from the vat, or treated with hypochlorite after it leaves the separator. Either method would considerably reduce the danger of airborne infection or infection from milk cans used to transport whey, but there are difficulties in technique and each would involve extra expenditure. Both methods are still under test.

The most important method of control is the propagation of starters free from infection. Unless the milk in the vat is heavily contaminated with phage the cheese-making process should be finished before the rate of acid production becomes dangerously slow. Nichols & Wolf(19) have shown that airborne phage can be destroyed by hypochlorite mists. They found this method

effective for maintaining the limited space of laboratories and starter rooms free from phage during the time required for the transference of cultures and preparation of starters, but it would be less useful in the larger volume of the cheese-making room, where the mists would need frequent renewal. Sutton (20) found that ultra-violet light destroyed bacteriophages provided that they were exposed in thin films of whey or dried whey.

Most bacteriophages were found to be resistant to 70° C. for 10–15 min.; few resisted 75° C. for 7½ min. (Nichols & Wolf (21)). The practice of sterilizing milk for the preparation of bulk starter by holding at 190–212° F. (87·75–100° C.) for ¾–1½ hr. thus allows a good margin of safety. Mattick, Nichols & Wolf (8) show how effective the provision of a separate and isolated starter room could be even when improvisations were all that were possible under wartime conditions. Laboratory control of the starter strains and daily tests for the presence in the whey of phages active against the strains in use allowed a change of starter before trouble became serious. A reserve of starters was available for use at short notice. Whitehead & Hunter (3) advocate the use in daily rotation of several different single-strain starter cultures susceptible to different specific phages. On any day the phage active against the starter culture in use would rarely be present in the milk in the cheese vats in quantities sufficient to retard cheese-making. Reviewing the work of the Dairy Research Institute, New Zealand, on bacteriophage infection in cheese manufacture, Whitehead & Hunter (22) give a clear account of the general trend of their researches during recent years. They indicate how trouble due to phage may spread as a result of airborne infection, and, once the infection is established in a dairy factory, how the cycle of infection may develop. They describe the methods used in New Zealand to combat the troubles.

Hunter's (12) researches on the growth of strains of *Str. cremoris* and their phages in milk indicate that the phage races are separate and distinct entities with growth conditions not necessarily identical with those of the organism on which they develop. The optimum and maximum temperatures of growth for a phage may differ widely from those of its homologous organism. This complicates still further the already involved problems of starter control and cheese manufacture, but it also helps to throw light on some hitherto unexplained happenings in cheese factories, such as the 'sensitiveness' of certain starter strains, the unequal capacity of strains to withstand heavy infection with their appropriate phages in the cheese vat at 37° C. and the greater chance of contamination of the bulk starter with airborne particles of certain phage races. When testing for the presence of airborne phages plates should be incubated at more than one temperature.

Later researches of Hunter (23) contribute to the solution of the problem of the differentiation of the lactic acid streptococci. Shattock & Mattick (24) have shown that, on serological grounds, *Str. faecalis* can be clearly differentiated from *Str. lactis* and *Str. cremoris*, but indicate that the last two fall into the same serological group (N). Hunter has shown that they may, however, be differentiated on their phage reactions. There was a definite tendency to strain specificity in the phages attacking the *cremoris* group. Each of the strains was susceptible to attack by only a few, sometimes by a single phage, while the strains of the *lactis* group were attacked by a variety. With two exceptions the phages attacking the *cremoris* types were distinct from those attacking the *lactis* types. None of them attacked the strains of *Str. faecalis* available for test. Hunter regards the results as justifying the differentiation of *Str. cremoris* and *Str. lactis* as two species.

Antibiotic substances

Experiments by Hunter & Whitehead (25) show that not only the presence of bacteriophage but also the use of 'non-acid' milk (milk which hinders the development of acid by a normally active starter culture in the vat) may be the cause of thin starter. The presence of inhibitory substances in the milk results in a prolonged lag period, but acid production proceeds and is not brought to an end by lysis of the bacteria as in the case of phage attack. The resistance to the inhibitory substance varies with the strain of starter organism. The amount of inhibitory substance depends on the extent of growth of the 'non-acid' streptococci; therefore fresh milk is less likely than

stored milk to contain inhibitory substances. It is advisable to use the freshest milk possible for starter making. Meanwell (26) found that the majority of 'slow' ('non-acid') milks were of poor quality, usually giving a methylene-blue test of less than 15 min. A toxin-producing acid-forming *Streptococcus* was isolated from many of the 'slow' milks. In one case the presence of these inhibitory substances in the milk was traced to the use of an unsterilized milking machine and inefficient cooling of the milk. The danger of slowness and even 'pack-up' in isolated vats due to the inclusion of, say, 5% of inhibitory milk is accentuated when the milk is pasteurized. The danger of 'slowness' due to inhibitory milk could be greatly mitigated by raising the standard of milk production and transport. Antibiotic substances have been extracted from cultures of inhibitory streptococci isolated from 'slow' milk. Oxford (27) has extracted a water-soluble protein, heat-stable in acid media, which is active against *Str. cremoris*, less active against *Str. lactis*, with a transient effect on *Staph. aureus*, and no action on *Bact. coli*. It has been tentatively called *diplococcin*. Mattick & Hirsch (28, 29) extracted a heat-stable substance which, although it contained only a small percentage of active substance, was of high potency and completely inhibited the growth of *Str. agalactiae* in a dilution of 1/640,000. Many groups of pathogenic streptococci were inhibited by it, also some species of *Bacillus*, *Clostridium* and *Lactobacillus*. Living cultures of the inhibitory *Streptococcus* produced sufficient antibiotic substances to destroy or render avirulent a culture of tubercle bacilli in milk.

Cheese starters

In general, starters for Cheddar cheese-making consist of pure cultures of *Str. cremoris* or *Str. lactis*, either as single strains or as mixtures. The selection and relative proportion of strains seems to be largely a matter of circumstances. On examining the component bacteria of nine starters which had been in use in factories for various periods, Rice (30) found that the relative proportions of *Str. cremoris* and *Str. lactis* varied widely. When the cultures were received at the laboratory *Str. cremoris* predominated in four and *Str. lactis* in five. Six of the starters were maintained in a dairy for 6 months, during which time they were propagated by the usual factory routine, i.e. in pasteurized milk and at fluctuating incubation temperatures. At the end of that time microscopical examination indicated that *Str. lactis* predominated in each of the starters, even those in which *Str. cremoris* had predominated at first. The experiments also suggested that slow starters may result from the replacement of active strains by slow-fermenting strains. He considers that this attenuation of the starter is the result of continued subjection of the strains to over-acidity, and to cultivation at too high temperatures. On the other hand, Johns & Bérard (31) found that over-ripening of starters did not lead to a reduction in the rate of growth or in the final acidity reached. Dahlberg & Ferris (32) showed that, although the transfer of starter cultures every third day instead of the usual daily transfer might not obviously affect the quality of the cultures, Cheddar cheese made with the latter developed a better flavour in a shorter time than cheese made with the former.

Deane and his colleagues (33, 34, 35) have studied the effect of certain pure cultures of bacteria on the flavour of Cheddar cheese. The cheese were made from raw milk, by the accepted commercial methods, and ripened in 12 oz. valve-type cans. In some batches a commercial starter was used, in others a culture of *Str. lactis*, and the pure cultures were used in conjunction with either. The cheese were judged for flavour at regular intervals. The addition of *Str. citrovorus*, *Str. paracitrovorus* and *L. bulgaricus* was of little value in improving the flavour of Cheddar cheese. The addition of an unidentified 'acidoproteolytic coccus' (isolated from a 4-year-old cheese) gave cheese of significantly better flavour than the control. In these experiments the bacteria were added to the cheese milk, and McDonald (36) also found that little improvement resulted from the addition of cultures of *L. casei* in this way. These organisms grow slowly in fresh milk and were therefore probably run off in the whey before they had begun to proliferate. When, however, a whey culture was sprinkled over the curd at hooping, or a milk culture over the curd at cheddaring, the organisms were retained in the cheese and hastened considerably the development of the mature flavour.

Pasteurized milk in Cheddar cheese-making

Early in the war, America was faced with a largely increased demand for long-keeping cheese of good quality. Unfortunately, much of the cheese offered was below the desired standard. As the result of experimental work already carried out under the auspices of the Bureau of Dairy Industry, it had been shown that pasteurization of the milk and the use of a controlled time schedule for the cheese-making process were important factors in the production of Cheddar cheese of uniformly high quality. An account of the researches on which the schedule is based is given by Wilson, Hall & Rogers (37). They point out that the quality of the milk is of primary importance. The detrimental effect of poor quality in the raw milk is only partially overcome by pasteurization, since although it is possible to destroy the bacteria, the changes which they have already brought about in the milk cannot be reversed. The rate of development of acidity should not be too great and should not proceed too far before the curd is milled. Such cheese can be safely ripened at temperatures high enough to allow the essential bacteria still to be active. The controlled system of manufacture sponsored by the Bureau was introduced into about 150 factories. Walter & Lochry (38) describe the working of this scheme and show that there was a striking improvement in the quality of the cheese, especially in those factories where much that had previously been produced was of a low grade.

It is fully recognized that cheese made from pasteurized milk is of a more uniform quality and free from many of the defects found in raw-milk cheese. Against this are the drawbacks that the cheese ripen slowly, and even when mature lack the full flavour of typical Cheddar cheese. America was committed to the production of Cheddar cheese from pasteurized milk, but it was also desirable that the ripening period should be shortened, and a fuller flavour be developed within this shorter period. A means of attaining this end was sought by raising the temperature of the ripening rooms. The view had hitherto been held that the lower the temperature of ripening the cleaner and better the flavour of the cheese. This was found still to hold for much cheese (that from raw milk in particular), but some pasteurized milk cheese could be ripened with safety and even with advantage at 60° F., although 70° F. was found to be too high. Cheese made from poor-quality milk, even after it had been pasteurized, could not be satisfactorily ripened at 60° F.; the quality was definitely inferior to that of cheese ripened at 50° F. When milk of a high quality was used there was little difference in the flavour or rate of ripening of the cheese, whether the milk was raw or pasteurized. A great deal of experimental work on this question has been done in the laboratory of the Bureau of Dairy Industry. A brief account of the bacteriological studies has been given by Tittsler, Geib, Sanders, Walter, Sager and Lochry (39), in which they indicate the changes in the number and types of bacteria during the ripening of the cheese. The co-operative experiments carried out under the direction of the Bureau, have yielded a mass of data on the bacteriological, chemical and physical changes in the cheese as well as on practical methods. Publication of these is promised, but meanwhile a preliminary report has been issued (40).

Flavour development in Cheddar cheese

The question of what is the most desirable flavour is difficult to decide—it is so much a matter of individual taste. Hansen (41) reports little difference in score between cheese made in Canada from raw or pasteurized milk and ripened at 40, 50 or 60° F., but there was a great deal of difference in the general descriptions of the flavour at the different ripening temperatures. In general, the flavour developed more rapidly at 60° F. Price (2) states that a study of consumer preferences made by the Wisconsin College of Agriculture indicates the preference of the public for mild, rather than strong flavours in cheese. On the other hand, in England most of the cheese available during the war was generally regarded as immature and decidedly lacking in flavour.

It is well established that hydrolysis of the fat is a factor in the ripening of cheese, and that the flavour depends in a large degree on the quantity and type of volatile acids produced. The destruction or weakening of the natural and bacterial lipases of milk during pasteurization may

in some measure account for the slow development of flavour in cheese made from pasteurized milk. Hammer and his colleagues at the Iowa Agricultural Experiment Station, have studied this problem (42, 43, 44). They estimated the amount of fat hydrolysis by the increase in the titratable acidity of the fat (obtained by pressing a mixture of cheese and sand and separating the fat by centrifuging the expressed liquid). There was an increase during the ripening of all cheese, but it was greater for raw-milk cheese than for pasteurized. Homogenization of the cream gave higher fat acidities, except when pasteurized cream was used. The effect of lipases from various sources was examined. The addition of pancreatin and mammary tissue increased the fat acidity, but the former gave an objectionable rancidity which tended to persist. The addition of rennet paste also increased the fat acidity in comparison with that of the control. Small quantities gave early rancidity which tended to disappear during later ripening; large quantities, especially when rennet paste was used as the sole coagulating agent, gave cheese which were rancid throughout the whole ripening period. Mulberry juice did not improve the flavour—small amounts had little effect, large amounts caused rancidity in the cheese. Although there was a general relationship between the acidity of the fat and the flavour, i.e. the higher the acidity the stronger the flavour, there was no true correlation. There was variation between the groups. This was true also for cheese from pasteurized milk. There was a steady and regular rise in fat-acidity, but the rate was not uniform between the different lots. No doubt the flavour of Cheddar cheese depends on many factors of which fat hydrolysis is only one, and this may explain why raw-milk cheese had a better flavour than pasteurized-milk cheese, even though there were not necessarily large differences in the fat acidities of the two types.

Hlynka, Hood & Gibson (45, 46, 47) have studied rancidity in Cheddar cheese (raw milk) and have found no evidence of the eventual disappearance of early rancidity which developed as a result of agitating the milk at a temperature of about 86° F. before making the cheese. Their experiments proved that a considerable amount of fat dispersion occurred when the milk was agitated, and a larger surface of fat was exposed to the action of the lipase in the milk, which may explain the development of rancidity in the cheese made from such milk. The score of the cheese varied but slightly from 2 weeks to 6 months of ripening at 40–46° F., and there was little tendency for the rancidity to increase or decrease. They concluded that the conditions in the cheese (*p*H, etc.) were not conducive to lipolytic action after the cheese was made. Data on the titratable acidity of the cheese fat (not given in the paper) are said to support this view.

Snyder & Hansen (48, 49) also studied the effect of homogenization of milk on the quality of the Cheddar cheese made from it. They made detailed analyses of the cheese at intervals of 3 weeks during a 24 weeks ripening period. The cheese from all combinations of homogenized milk and cream, except pasteurized homogenized milk, were rancid, and the rancidity persisted throughout the ripening period. Homogenization did not materially affect the distribution of the soluble nitrogen in the cheese serum. On the basis of taste and aroma, the quality of cheese made from homogenized milk was lower than that made from unhomogenized. It is obvious that the problem of making a full-flavoured Cheddar cheese from pasteurized milk is not yet solved.

In the above remarks rancidity has been considered as being mainly due to the action of milk lipase, but bacterial enzymes may also produce a similar defect. The most important organisms in this connexion are the butyric acid-producing bacteria. Castell & Irvine (50, 51, 52) attempted to isolate and enumerate the butyric acid anaerobes in rancid and normal cheese. The numbers found were so small in comparison with those found in a true 'stinker' that it seemed doubtful whether they were the actual cause of the trouble. It was noted, however, that the rancid cheese had a more varied flora and contained more iodophilic clostridia. They found no evidence of multiplication, sporulation or germination of spores of butyric acid anaerobes in Cheddar cheese made from milk inoculated with these bacteria, nor was there any development of rancidity. When good-quality and rancid cheese were inoculated into a sucrose cream medium there was no evidence of greater lipolytic activity in the rancid samples. Since neither the presence of butyric acid-producing bacteria, nor greater lipolytic activity could be demonstrated in the rancid cheese, the authors were inclined to agree with the views of Hlynka, Hood & Gibson that

the determining factor in the development of rancidity is the treatment which the cheese milk undergoes. Any factor which tends to desurface the fat globules favours the development of rancidity. The butyric acid-producing bacteria are a cause of abnormal gas production in Swiss cheese and will be referred to again in that connexion (p. 187).

Though, strictly speaking, it may be encroaching on the chemical review, it seems appropriate to mention here methods of determining volatile acids as a measure of lipolytic acitivity. The simplest method is that of direct steam distillation which has been used by Irvine (52) for determining the lipolytic activity of rancid cheese by estimating the change in volatile acidity induced by planting a sample of the cheese in a sucrose cream medium. Only a portion of the acids are recovered by direct steam distillation of cheese, and Smiley, Kosikowsky & Dahlberg (53) have elaborated a method in which the acids are extracted from the cheese by shaking with ether, washed out with dilute NaOH solution, and steam distilled from these washings. The method is designed for general cheese studies. Lane & Hammer (43) followed the course of fat hydrolysis in cheese by titrating the fat obtained from the press juice of a mixture of sand and cheese. Estimations of butyric acid may be useful in problems concerning rancidity in cheese, but values obtained by this method may be misleading, since only about 40% of the free butyric acid will be dissolved in the fat, the remainder being held in the serum (Hiscox, Harrison & Wolf (54)). Lind (55) has used estimations of volatile acids to determine whether propionic acid- or butyric acid-producing bacteria were responsible for faults in Swiss cheese. For this purpose he adapted the method of Van Beynum to the direct fractional distillation of cheese. As each fraction of 30 ml. is collected it is replaced by 30 ml. of hot distilled water and the fractionation continued. The first fraction is redistilled and the process continued until too little acid is left in the liquid to be distilled. The type of acid is determined from the percentage ratio of the titre of the first fraction of a series to that of the first fraction of the preceding series, and from the quotient of the titre of any fraction in a series and the titre of the first fraction in that series. Comparison is made with standard curves. There are inaccuracies in the method, but it has proved itself valuable for the purpose for which it was devised.

Differences in salt content in different parts of the cheese may affect the uniformity of development of bacteria while the cheese is in the press, when, as has been shown by Hoecker & Hammer (56, 57), the salt content is higher at the surface than at the centre. These differences tend to disappear until, about a day after the cheese has been removed from the press, the distribution of salt is almost uniform throughout.

Abnormal gas-production in cheese

The presence of coliform organisms in Cheddar cheese and their role in the 'blowing' of cheese of this type have been studied by Crossley (58, 59) and Yale & Marquardt (60, 61), who came to similar conclusions. Cheese, whether made from raw or pasteurized milk, invariably contained coliform organisms, in the latter case by contamination from equipment in spite of all precautionary measures. The presence of coliform organisms did not necessarily foretell blowing of the cheese. Other factors, such as numbers of organisms and the consistency of the curd, were involved. Crossley found that strains of *Bact. coli* I survived longer than other strains and tended to predominate in the stored cheese, but in the cheese examined by Yale there was no evidence that any particular type lived longer than the others. Crossley distinguished two types of blowing. The normal type developed gradually throughout storage and was generally due to strains of *Bact. coli* I. The less common type was a rapid and violent fermentation occurring within a few days of leaving the press or even while the curd was in the vat. A mixture of strains was found in these cheese but *Bact. aerogenes* was always present. Yale found that a high acidity in the vats favoured the starter at the expense of the coliform organisms, but that a high temperature favoured the latter. In practice the use of active starters and as low a cooking temperature as possible should help to control gas production in Cheddar cheese. Too high an acidity, however, while suppressing coliform organisms, will cause other faults.

Coliform organisms are the cause of blowing in other types of cheese, the 'early blowing' of Swiss cheese for example. Such cases have been described by Hostettler, Binz & Sahli(62), van Beynum & Pette(63) and Dorner(64). In the case described by Dorner the first sign of blowing did not appear until 18 days after the manufacture of the cheese. A strain of *Bact. coli* was isolated which produced gas in a medium containing sodium lactate, and Dorner suggests that fermentation of lactates in the cheese by coliform organisms was the cause of the defect. In effect this implies that coliform organisms may be a cause of 'late blowing' in Swiss cheese, a defect which is normally due mainly to the fermentation of lactates by butyric acid bacteria.

Hostettler(65) traces the history of Emmental cheese and indicates changing views as to what constitutes a desirable size of eyes. Demands vary with the country, but generally the preference is for larger eyes than formerly. Normally the eyes are round, but with abnormal fermentations they may be small and irregular in shape, or large and torn. Blowing of cheese, with abnormal eye formation, a major defect of Swiss cheese, has been studied in European countries. Four types of organisms that may be responsible are listed by van Beynum & Pette(63): (1) coliform organisms which cause the 'early' blowing, (2) butyric acid bacteria, (3) *Lactobacillus bifermentans* (rarely found), and (4) the propionic acid bacteria, all of which cause 'late' blowing. Weiser(66), in America, cites *Torula cremoris* as being present in large numbers in blown but not in normal cheese, and concludes they are a main cause of the defect. Van Beynum & Pette(63) isolated the bacteria from the cheese on special media and identified them by their biochemical reactions, especially the gas produced by fermentation of a lactate medium. Lind(55) has used his distillation method to identify the volatile acids produced and so determine the type of fermentation that has occurred in the cheese. If coliform organisms are the cause of blowing, the acids produced will be mainly acetic and propionic, as in a normal cheese; if propionic acid bacteria, there will be excessive quantities of propionic acid and a high *p*H; if butyric acid bacteria, there will be much butyric acid and a high *p*H, frequently higher than 6·0. He found that blowing due to excessive numbers of propionic acid bacteria was common, occurring in about half the cheese he examined. It was infrequent, however, in the Danish Swiss cheese. Although this somewhat empirical method does not give strictly accurate data, it gives a rapid means of determining the type of fermentation in a cheese, and since the test can be applied within a few weeks of making, it may be possible to correct, or at least to palliate, the fault.

The butyric acid bacteria gain access to the milk from a variety of sources, of which silage appears to be one of the most frequent. Hostettler, Sahli & Binz(67) examined 829 samples of milk from various sources. They found that where silage was not used as fodder the butyric acid bacteria were rarely found in the milk; where silage was used most of the samples were infected with these bacteria. They found no exact correlation between the numbers of butyric acid bacteria in the milk and the percentage of hydrogen in the gas from the cheese, but of seventy-one cheese made from milk free from these organisms 94·4% contained not more than 0·3% hydrogen. The gas from eleven cheese made from milk containing many of the organisms contained 36–38% hydrogen. Since the organisms may be present in the spore form they are difficult to destroy by heat treatment, but Sjöström(68) has shown that their vulnerability is increased by heating at a low *p*H in strong solutions (more than 4%) of NaCl. Zollikofer & Richard(69) suggest the possibility of producing unblown cheese from silage milk by the use of 3% salt, but this has still to be tried in commercial practice.

The *p*H of the young cheese also influences the type of fermentation. Virtanen(70) suggests *p*H 6·35 at dipping, below 5·30 on leaving the press, and 5·5 during ripening. This agrees very closely with the acidities that Benedict, Frazier, Feutz & Swanson(71) state will give the best cheese. They and other American workers(72) also stress the importance of using cleanly-produced milk; active starters are essential. Bartholomew, Barber, Price & Frazier(72) found that *L. bulgaricus* gave the best results when the acidity of the culture ranged from 0·95 to 1·0%. For *Str. thermophilus* 0·65–0·74% was best. They found no improvement when *L. casei* was used in addition to the other two starter bacteria. Voss & Frazier(73) found that *L. bulgaricus* gave better cheese when grown at 76° F., but *Str. thermophilus* was more active when grown at 86·5 or 99° F.

Fisker(74) has examined 'Ektol', a preparation which claimed to hasten the ripening of Swiss cheese so that it would be ready for use in 5–6 weeks. He found no advantage in its use for Swiss or Gouda cheese. He identified it as a culture of *Thermobacterium helveticum* (*L. helveticus*).

Lind(55) has made a special study of the propionic acid bacteria grown alone and in conjunction with other bacteria. Van Beynum & Pette(75, 63) found that although these bacteria were always present in Dutch Gouda and Edam cheese, they multiplied only slightly in them and were of no importance in the production of good cheese. A late fermentation, whereby a few small holes are produced, is desirable, but too great a proliferation of the organisms may cause faults. The growth of the bacteria can be controlled by adjustments of the salt and moisture content, the ripening temperature and, above all, the *p*H of the cheese which should not be above 5·1 at 6–8 days after manufacture.

Brick and Limburg cheese

A study of the bacterial flora of the interior of Brick cheese has been made by Foster, Garey & Frazier(76, 77, 78) and of the smear by Langhus, Price, Sommer & Frazier(79). *Str. lactis* was always found in the interior of the cheese. When *Str. lactis* was used as the starter it predominated; when *Str. thermophilus* was used as starter, it died out rapidly and *Str. lactis* eventually predominated. The chief function of these organisms appeared to be acid production during manufacture. Some of the experimental cheese were made by means of the 'washed-curd' method, in which part of the whey in the vat was replaced by water, so lowering the concentration of lactose in the whey left in the cheese. It was thus possible to make cheese containing the maximum legal water content and yet avoid the excessive acid production which leads to poor texture and other ripening faults. The 'mild-wash' method removed about 35% of the lactose and the remainder was fermented, usually within 1 day, giving a *p*H of about 5·00. In addition to *Str. lactis* which was present in all the cheese, *Str. thermophilus* was found in those cheese to which it was added as starter, and *Str. bovis* more particularly in cheese made from pasteurized milk. *Str. liquefaciens* occurred irregularly and seemed to be connected with the development of undesirable flavours. *Str. faecalis* was often present in considerable numbers, but its function in the cheese was not determined. Of the lactobacilli, *L. casei* was the most important and is probably necessary for the normal ripening of Brick cheese. This organism was always found in raw-milk cheese, but its development was often restricted in pasteurized-milk cheese, and in its absence the cheese lacked body and flavour. *L. brevis* was found frequently and *L. lactis* occasionally, but they are probably of minor importance. Gas production due to anaerobic spore-forming bacteria was found only in cheese with a relatively low acidity (above *p*H 5·20). They grew readily in the cheese in which *Str. thermophilus* had been used as starter, and were favoured by the use of pasteurized milk and the washed-curd technique. Discussion of defects due to coliform organisms was reserved for another paper. The satisfactory development of the 'smear' of Brick cheese depends on the growth in succession of particular micro-organisms. Large numbers of yeast-like organisms develop first, followed by micrococci and rod-shaped organisms of the *Bact. linens* type which have a considerable acid and salt tolerance. The development of this smear reduces the acidity of the surface layers of the cheese, and though the rate of decrease is greatest during the period of most active growth, a wide difference between the *p*H of the interior and surface layers persists throughout the ripening period. There was, on the other hand, no marked difference between the water- and alcohol-soluble nitrogen compounds of these layers, and no measurable data could be found to suggest that there was any ripening 'from the outside'. The role of the micro-organisms of the smear appeared to be limited to the surface layers, but the pungent flavour of the interior is probably partly due to the diffusion of substances produced at the surface. The surface flora of Limburg cheese is very similar. Yale(80) found that yeasts, mainly white torulae, and *Oospora* develop first and reduce the acidity of the surface layers to a *p*H suitable for the growth of the rod-shaped bacteria. *Bact. linens* was found in large numbers, but did not predominate and was outnumbered by another group of alkali producing rod-shaped

bacteria which did not liquefy gelatine or produce a pigment. A study of the two main types is being undertaken.

A description of *Bact. linens* has been given by Albert, Long & Hammer (81, 82), who developed a special selective cheese agar for its isolation. The organism was isolated from a great variety of sources, including the surface and interior of many types of cheese, butter, milk, cattle fodder, and the air of dairy plants and cheese factories, but it was rarely found in soil. The organism was strongly proteolytic but not lipolytic. It was also able to produce volatile acids from alcohols. Colour production was improved by the addition of peptone or a mixture of peptone and casein to tryptone glucose extract agar, which suggests that the colour on the cheese is influenced by the nature of the protein breakdown. *Bact. linens* grew at *p*H 9·8 and 6·0 but not at *p*H 5·0, and rapidly disappeared in milk in the presence of actively growing cultures of *Str. lactis*. It was readily destroyed by heat, but tolerated 15% salt in broth and skim milk.

Blue-veined cheese

As a result of years of research blue-veined cheese of the Roquefort type can now be manufactured on a large scale in America with a considerable assurance of a uniform product of high quality. Fabricius & Nielsen (Iowa State College Research Foundation) (83) have patented a process in which homogenized whole milk is used for its manufacture. Berger (84) describes a medium for the cultivation of the Roquefort mould, avoiding the use of agar, which at the time was almost unobtainable. Milk is coagulated with *Thermobacterium yoghurt* (*L. bulgaricus*), spread in dishes, sterilized and dried sufficiently to make it firm. The plates are inoculated by means of air distribution of the spores in a special chamber. Following up his earlier work on the gas requirements of mould, Golding (85) found that *Penicillum roqueforti* grew better than most other common dairy moulds in high concentrations of CO_2. Applying this knowledge to dairy practice he has developed a method of manufacturing blue cheese in which the curd, after draining in long hoops of the same diameter as 8 oz. cans, is cut into pieces the same height as the cans, salted, drained, placed in the cans and the lids loosely fitted. The cans are then placed in a cold store at 30–40° F. in an atmosphere containing 30–40% of CO_2. When the desired degree of mould growth has been reached, in 6–10 weeks, the cans are vacuum sealed and placed in the ripening room at 50° F. for several months. Mould growth is encouraged and controlled and contamination is avoided. Thibodeau & Macy (86) have studied the protease and lipase of *P. roqueforti* using a mycelium powder as the source of the enzymes. The tests on the protease indicate that it differs from animal proteases and is of the trypsin type. The fat-splitting enzyme is not a true esterase but a lipase. The addition of animal enzymes (which differ from those of the mould) to shorten the ripening period may therefore bring about undesirable changes. The addition of mycelium powder to the curd at the time that the mould powder was added shortened the ripening period by about 5 months. Protease was not released into the culture medium until the mycelium was beginning to disintegrate, but small amounts of lipase could be detected a few days earlier. This is in contrast with many other moulds. Attempts to extract the enzymes from the mycelium have so far been unsuccessful. Blue cheese are often disfigured with a red mould growth. Hammer & Gilman (87) have isolated this mould and identified it as *Sporendonema casei* (frequently known as *Oospora crustacea*). Todhunter, Roderick & Golding (88) found no evidence that mould growth in Roquefort type cheese increased the vitamin A value.

Surface moulds and insect pests

Prevention of the growth of moulds and of insect pests on the surface of ripening cheese would eliminate a source of serious loss to the industry. Sutton (20) found the destruction of mould spores on moist and dry surfaces by exposure to ultra-violet light sufficient to suggest that this might provide a useful method of control in cheese rooms. Smith (89) found that the amount of radiation from low-pressure ultra-violet lamps required to destroy spores of *Penicillum roqueforti*

was 40–80 times that required to destroy *Bact. coli*. Six 15 W. cathode lamps placed in a cheese room 15 ft. 17 in. × 12 ft. 2 in. × 11 ft. 3 in. did not control mould growth, nor did the exposure of cheese, before paraffining, to 19 min. irradiation by three lamps at 10 in. from the surface. A further irradiation for 10 min. after paraffining did not increase the effect. Materials commonly used for coating had a marked screening action on the 2537 A. ultra-violet light. Platon & Thomé (90) tested the effect of irradiation with Sterilamps, wave-length 2537 A., on pure cultures (grown on plates of Difco malt agar containing an extra 1% of agar) of moulds and yeasts common on cheese or in cheese stores. Although direct irradiation for 2 days did not destroy all spores in fully developed cultures, growth and spore development were arrested in immature cultures, and growth prevented in freshly inoculated plates. Cheese less than a month old, and freshly paraffined, were exposed to continuous irradiation in previously disinfected ripening rooms. At 4 weeks all irradiated cheese were mould free, and 6·7% were still free at 6 weeks, although all the control cheese had become more or less mouldy. These experiments were temporarily discontinued because of the difficulty of obtaining sufficient Sterilamps under war conditions, but experiments had already indicated that chemical treatment could be an effective protection against mould growth.

Platon, Emgard & Olsson (91) treated cheese surfaces with benzoic acid and methyl *p*-hydroxybenzoate (Nipagin). These are insoluble in water and alcoholic solutions must be used. Dipping the cheese into 10% solution of benzoic acid kept it free from mould growth for several days, although the controls were overgrown in 3–4 days. In practice the cheese were dipped in a 25% solution as they came from the press or when dry after brine salting, and waxed within a week. They were wiped with a cloth dipped in the solution before rewaxing after about 2 weeks. Methyl *p*-hydroxybenzoate is less volatile than benzoic acid, and one dipping was sufficient to keep the cheese free from mould for about 4 months. Dahle & Warren (92) found that adding calcium or sodium propionates or propionic acid to cheese curd with the salt or as a dilute solution in the form of a spray would prevent the growth of blue mould in Cheddar cheese even when blue-veined cheese were made in the same factory. Mann & Frazier (93) found that these propionates inhibited the growth of *Bact. coli* and *Alkaligenes viscosus* in milk products. They had little effect on *Oospora lactis* but considerably reduced the growth of *Str. lactis*. If used in cheese-making as preventatives of various faults, these chemical preservatives would therefore need to be handled carefully. At present, however, regulations would not allow their addition to milk or dairy products.

Other chemicals are needed to combat the insect pests which attack the surface of ripening cheese and of which cheese mites offer perhaps the most serious problem. A detailed study of the mites is being made at the Cawthron Institute, N.Z. (94, 95, 96), where four types have been found. The constitution of cheese mite colonies differed specifically in different factories and the factors determining the incidences of each species are being sought. Waxes and dusts offered a possible means of protection and control, but ammonia and CO_2 were unsuitable fumigants. Methyl bromide was effective but had disadvantages. Dichloroethyl ether was found to be highly toxic to the common cheese mite (*Tyroglyphus siro*) even at low concentrations. No foreign flavour developed in the interior of cheese kept 6 weeks in an atmosphere saturated with dichloroethyl ether. Moreover, timber (shelving, etc.) treated with this chemical had a protective influence on cheese in contact with it. It is reported from the Dairy Research Institute, N.Z. (97), that ammonia destroys mites, but is not an effective fumigant in large cheese stores. They consider methyl bromide is more effective. Roehm, Stenger & Shrader (98) confirmed earlier findings that methyl bromide did not penetrate more than 0·25 in. into solid cheese. With normal commercial fumigation (1–5 lb. per 1000 cu.ft.) all the absorbed methyl bromide was lost from the cheese within 3 days. The fumigant penetrated wax but not cellophane with a rubber-like lining. Outbreaks of dermatitis, due to irritation set up by mites, amongst workers unloading imported cheese, and amongst girls handling cheese that had been stored for a long time are reported by Dowling & Thomas (99). Thompson (100) has given an account of insect pests (including flies, cheese mites and cheese skippers) of cheese and butter factories and indicates methods of control.

Vacuum-packing of cheese

The ripening of cheese in vacuum-sealed tins avoids the use of the expensive and sometimes defective valve-vent can, and may also avoid the bulging due to the pressure of gases accumulating in air-sealed cans. Dorn & Dahlberg(101) have shown that the gas evolved by Cheddar cheese ripened in cans consists of carbon dioxide with traces of hydrogen, and that evolved by Limburg cheese after a preliminary ripening was a mixture of carbon dioxide with 5–10% hydrogen. Dahlberg & Marquardt(102, 103) found that a vacuum of 20–25 in. rendered innocuous the gas evolved during the ripening process (especially when gas-producing organisms had been destroyed by pasteurization of the cheese milk). The prevention of bulging of the tins could be made doubly sure by enclosing a little calcium or magnesium hydroxide in the tins to absorb CO_2. With this addition the ripening could be prolonged for a year or even more. Cheddar cheese made from finest quality raw or pasteurized milk ripened slowly at 40° F., but the flavour developed much more quickly at 60° F. Cheese of the Limburg, Tilsit, Camembert and Brick types could be ripened satisfactorily in vacuum-sealed cans if the canning was delayed until the preliminary surface ripening was well advanced (in 2–3 weeks). Golding(104, 105, 106) has also examined the use of vacuum-sealed cans for the ripening of Cheddar cheese. Preliminary experiments showed that there was less loss of vacuum when the cheese was made from pasteurized milk, and because, in addition, greater uniformity in the cheese was probable, this type was used in all further experiments. Records of the vacuum inside the tins were made at frequent intervals during ripening of cheese at temperatures 42, 53 and 63° F. Up to a period of 21 days (the experiment was still in progress) the loss of vacuum was greatest in the cans held at 63° F. Cans of mature commercial cheese were held at 51·9, 61·6, 67·5 and 73·6° F. for 50 days to simulate conditions of retail and domestic storage. There was little change in the vacuum in the tins held at the lowest temperature, but at the highest there was a rapid loss of vacuum in some of the cans and two had bulged. Mature vacuum-packed cheese should be held at 50° F. or below until it is consumed. Golding(107, 108) showed that when the starter produced acidity rapidly during the cheese-making process the subsequent gas production was slight, but if the starter produced little acid, sufficient gas to bulge the ends of the tube might be produced on incubation at 40–50° F. Different commercial starters materially affected the rate of production of gas. Combining a *L. bulgaricus* starter with the ordinary lactic starter reduced the amount of gas produced and lessened the danger of bulged tins. The higher the proportion of *L. bulgaricus* culture in the mixed starter the less was the amount of gas produced. The flavour and texture of the mature cheese were not, however, those of typical Cheddar cheese.

III. BUTTER (Bibliography only)

Three comprehensive reviews deal with factors influencing bacterial growth in butter(1), the bacteriology of butter cultures(2), and the action of butter cultures in butter(3).

Sundry papers deal with:

(*a*) General aspects of butter cultures, especially the development of aroma(4–17).
(*b*) Development of surface taint and other off-flavours(18–29).
(*c*) Black discoloration(23, 30).
(*d*) Coliform organisms(31, 32, 33).
(*e*) Detection of lipolytic organisms(34, 35).
(*f*) Yeasts and moulds(36–41).
(*g*) The visual mould test(42–49).
(*h*) Prevention of surface mould growth(50, 51).

IV. PASTEURIZATION AND OTHER PROCESSES

(*a*) Pasteurized milk

Three books view the problems of pasteurization from different angles. Wilson (1) deals mainly with the purely scientific and public health aspects, Hill (2) is chiefly concerned with general plant design, operation and control, and Enock (3) views the problem as a dairy engineer, interested particularly in the design and construction of pasteurizing plant. A chapter in Microbiology of Food by Tanner (4) is devoted to pasteurization.

Many of the scientific papers available for review in this section are concerned, directly or indirectly, with high temperature-short time pasteurization, which, because of its many advantages over the holder process, has continued to gain acceptance. The margin of safety in the destruction of pathogenic organisms is narrower than in the holder process, but with developments in the design of sensitive automatic control instruments the danger of the passage of underheated milk into the forward flow becomes increasingly remote. The substitution of automatic control for the human factor calls for a different attitude of mind towards the pasteurizing process. This was recognized by the authorities of Michigan, and when H.T.S.T. pasteurization was legalized in this State in 1945, the members of the dairy industry were invited to a Conference at which various aspects of the process were discussed by experts. The papers read at this Conference have been collected in a *Special Bulletin* (5).

There is not, however, universal agreement on the wisdom of accepting H.T.S.T. pasteurization as an alternative to the holder process. Doubts as to the safety of a process which does not include a positive holding time have been expressed by Carter (6) and by Enock (3), who, himself, advocates the 'in-bottle' process as the safest method. Controlled experiments in the laboratory and with H.T.S.T. plants under experimental conditions have shown that the process allows a good margin of safety for the destruction of pathogenic organisms. No new data are available, but evidence for this claim will be found in earlier reviews (7) and some references are given by Hiscox (8). In commercial operations, however, conditions are not always ideal, and there is a possibility that, although automatic controls are compulsory in each plant, they could become ineffective through misuse. The results of a survey of pasteurizing plants in the West Midlands of England made by Clegg & Lomax (9) during 1945 suggest that this risk is not great. Their findings do not support the view, frequently expressed, that the holder process is a surer safeguard for the milk supply. During this period 645 samples of milk were tested from fifty-eight plants, twenty-nine of which used the Holder and twenty the H.T.S.T. process. Of the samples from the latter plants 1% gave a positive phosphatase test, but 5·4% of the samples from the holder plants were positive. In this area, therefore, the H.T.S.T. process gave the more reliable results. Moreover, the samples had a slightly longer average keeping quality, and a higher percentage was free from coliform organisms. The authors ascribe these favourable results to the use of precision control instruments and the greater ease of cleaning in the H.T.S.T. plants.

Progress in plant design. Progress in the construction of new plant has been in the direction of improvements in existing types rather than in the development of new designs. Numerous patents have been granted for improvements in heat-exchange apparatus, flow-diversion valves and plant for 'in-bottle' and H.T.S.T. pasteurization.

A new English plant for H.T.S.T. pasteurization, developed by the G.E.M. Company (10), is of the open-surface type, and during the final heating and cooling stages of the process, the milk flows by gravity over the outer surface of a series of tubes through which the heating or cooling medium flows. The holder is in the form of a trough.

The plates of the latest A.P.V. plant (11) have an improved form of groove which increases the rate of heat transfer. A new design of flow-diversion valve is included. Kossirev (12) described a plant in which the milk passes through the space between two concentric tubes as in the Stassano machine, but in this space is wound a spiral of 3·5 mm. wire. It is claimed that this plant is 3–5 times as efficient as the Stassano plant. The Trumbull Electro-pure pasteurizer is described by Palmer (13). Changes in the design of the plates and holders in the Kolding (14, 15, 16, 17) plate

pasteurizer have made for greater efficiency. Reports on the construction and performance of the Kolding(15, 16, 17), the Silkeborg(18, 19), the Spirala(20) and the Super-plate(21) pasteurizers have been issued by the Danish Dairy Research Station.

Leete(22) has described an electrically heated tank in which small supplies of milk can be pasteurized by the 'in-bottle' process. Thin-walled, over-sized bottles are used to allow for expansion and ensure uniform heating by convection. Although in some types of plant electricity serves as the heating medium either indirectly as above, or directly, as in the Electro-pure plant, steam is more generally used. Gillespie(23) has pointed out its advantages, the greatest of which is, perhaps, its lower cost. Steam is in constant use for various purposes in most dairies, and it may be possible to instal H.T.S.T. plant without the necessity for increasing the boiler capacity. Usually steam is used indirectly, i.e. to control the temperature of the heating water, but it may be injected directly into the milk stream in cases where dilution of the milk is not a disadvantage. Its direct use for pasteurizing cream for butter-making and dairy by-products for stock feeding was mentioned in the last review(24). Lazarus & O'Brien(25) describe a plant in which milk for Cheddar cheese-making is pasteurized by the direct injection of steam. Up to 18% of water may be added to the milk by this method, but means have been found of correcting the reduction in curd strength resulting from this dilution. The cheese were of good flavour, with body and texture similar to that of washed-curd cheese. Minor modifications in manufacture, such as the use of slightly more rennet and a higher cooking temperature, were necessary, but on the whole manufacture was not difficult and the cheese appeared to ripen normally. Marquardt & Yale(26) reported favourably on cheese made by this process.

Following up results of laboratory experiments Dahlberg, Holland & Miner(27) have tested the 'Quick-time' pasteurization on a commercial scale. The milk was passed through a series of twelve tubes in which the milk was heated from 140° F. to the desired temperature in the 4th and 5th tubes, cooled to 140° F. in the 6th, and to a lower temperature in the remainder. The time during which the milk was above 140° F. could be controlled and recorded. In the tests these time intervals varied from 5 to 24 sec., and the pasteurizing temperatures ranged from 177·5 to 169° F. At these temperatures, the margin between the temperature at which impairment of the creaming power began and the temperature that gave milk with a negative phosphatase test appeared to be greater than that at the lower temperatures used for H.T.S.T. pasteurization. This temperature spread was found to be 5° F., so that a tolerance of ±2·5° C. might be allowed. In a general article on H.T.S.T. Weinreich(28) remarks on the change from tubular equipment, extensively used at first, to the modern plate pasteurizer. Now that plates of various designs can be readily pressed or milled from stainless steel(16), their relative cheapness and greater efficiency of heat transfer have brought them into favour, and plant of this type has largely replaced the tubular. Weber(29, 30) has discussed the construction and equipment of a milk-pasteurizing plant, dealing in particular with those phases which have a direct bearing on public health. H.T.S.T. plants are complicated and methods of testing are far from simple (he deals with these in some detail), and he advocates standardization of plant design and installation, and a search for the best methods of testing, which should then be accepted as a standard.

Control instruments. The effectiveness of the H.T.S.T. process depends on precision timing and the sensitivity of the control instruments. Dupen(31) discusses the control system of a well-designed plant in which variations in steam pressure, incoming milk temperature, milk flow, water flow and heat exchange are reduced to a minimum. Olson(32) also gives a detailed description of the automatic controls of an H.T.S.T. pasteurizing plant. He stresses the need of routine checking at all vital points as a precaution against breakdowns and other failures. Holt(33) considers that each unit should be thoroughly checked before it is approved and frequently thereafter. He deals with the various points that should be tested and concludes that, with proper supervision of each installation, the H.T.S.T. process is safer than the holder method.

The question of control instruments is a highly technical one and belongs to a technological rather than to a bacteriological review. But since the effectiveness of the destruction of bacteria is so intimately connected with the accurate control of the time and temperature conditions,

some relevant points must be included here. Cuttell(34), after describing the adjustments and controls that are needed for the satisfactory performance of the plant and of the points at which they are applied, stresses the close connexion between plant control and bacteriological results, and again emphasizes the need for the training of operators qualified to understand and apply the broader aspects of dairy technology. One essential control of H.T.S.T. plants is the checking of the holding time. Two methods that are in general use have been referred to in previous reviews, but they have been tested and again reported on by Fay & Fraser(35), who found that the dye method checked well with the conductivity method using salt solution and ammeter. The form of electrode is described, but adjustments of sensitivity are necessary to meet variations in local water supplies used in the timing tests. A higher reproducibility was attained when an automatic electric timer was used instead of the usual stop-watch. A simple method of timing the Electro-pure pasteurizer is given. Dummett & Mongar(36) criticize both these methods on the grounds of insensitivity and the necessity of injecting comparatively large volumes of liquid, which may disturb normal flow conditions. In their method 5 ml. of a saturated solution of nickel chloride is injected by means of a special quick-acting gun through a boss suitably placed at the inlet to the holder. A special sampling cock adjusted to deliver 75 ml. per second is placed at the outlet. Samples are collected into calibrated test-tubes containing dimethylglyoxime, square-topped and just touching at the tops so that a continuous series of samples can be taken without loss. A yellow colour due to the interaction of the two reagents indicates the presence of nickel in the sample and a sensitivity of 1:100,000 is claimed. Replicate tests should not differ by more than 1 sec.

These tests are all made in a water stream and cannot be applied to the actual milk flow. In this connexion mention may be made of the method used at the Danish State Experimental Dairy, in testing the efficiency of the milk flow through the various sections of a pasteurizer. A three-way cock is fixed immediately before the plant, which is filled with skimmed milk of a known fat percentage. By turning the cock whole milk, of which the fat percentage is known, is allowed to follow the skimmed milk through the plant. Samples are taken every 2 sec. at suitable points, e.g. before and after the holder, and the fat percentages determined. The time taken for the full transition from skimmed milk to whole milk at any point is a measure of the efficiency of the flow up to that point. From these figures the longest and shortest times taken by the milk particles to pass through any section of the plant can be determined. The reports (14, 15, 16, 17, 18, 19, 20, 21) show that while the difference between the longest and shortest times in the heating section appears to be consistently about 2 sec., it varies from 18 to 4 sec. in holders of different construction.

Flow diversion. The regulations for the operation of H.T.S.T. plants require that a flow-diversion valve shall function when the temperature in the holding section falls below a specified value. With a holding time as short as 15 sec. a rapid response to fluctuations in temperature in the milk stream is essential, and, moreover, small changes in temperature must be recorded. These requirements are much more stringent than those demanded of the control recorders used in the holder process, but in England, at least, the limitations imposed by war conditions often compelled the use of identical instruments in both processes. Hall(37, 38) examined mercury-in-steel and vapour-pressure thermometers used in the control of pasteurizing plants, and found that neither satisfied the demands of the H.T.S.T. process in both speed of response and accuracy. The lag curves for the mercury-in-steel thermometer closely approximated straight lines, the slope of all giving a value for lag of 3·0–3·2 sec. The curves for the vapour-pressure thermometer were far from straight and gave values varying from 2·9 to 3·9 sec., a result which is probably due to the presence of both liquid and vapour in the bulb. On the other hand, the vapour-pressure thermometer offered a better response to small changes in temperature, though even with this instrument there was a time delay before there was any response at all. With the mercury-in-steel thermometer, the temperature drop was in some cases completely ignored. Hall found that thermocouples used in conjunction with a d.c. amplifier-recorder system offered considerable advantages in accuracy and speed of response, and showed promise of satisfying the needs of the dairy industry. A thermo-

couple with its lag constant of 0·2 sec. will give an almost ideal response, and the accuracy of temperature measurement and control could, by its use, be made four times as great as that now attained in general practice. Goodman & Hotchen (39) describe a new mercury-in-steel thermometer in which the lag coefficient has been reduced to less than 0·5 sec. The bulb is made of stainless steel, but the space inside is almost filled with a practically non-expansible 'Invar' core which is hollow, evacuated and hermetically sealed to reduce its heat capacity to a minimum consistent with adequate mechanical strength. There is a clearance of a few thousandths of an inch between the core and the stainless steel envelope, and this space is filled with mercury which, in this thermometer, serves merely as a medium to transmit changes in the volume of the envelope to the Bourdon tube actuating the flow-diversion valve. The action is the reverse of the usual, since a rise in temperature reduces the pressure in the Bourdon tube. The outstanding faults of the usual type of mercury-in-steel thermometer are eliminated and an accuracy of $\pm 0{\cdot}3°$ F. and a lag coefficient of 0·3–0·4 sec. according to the rate of flow past the bulb are claimed for this thermometer. Yoxall (40) discusses the vapour-pressure thermal system in the control of H.T.S.T. pasteurizing plant. He considers that this system has a sufficiently high rate of response to enable it to follow any temperature deviation that can occur on an H.T.S.T. plant. Caffyn (41) views the problem rather more from the academic standpoint. He does not deal with the difficulties or the advantages of any particular type of plant, but discusses the principles underlying the control at each point. The last four references are to papers read at a joint Conference of the Society of Dairy Technology and the Society of Instrument Technology held in London in May 1946. They and the discussion which followed (42), also a later paper by Matthews (43), indicate the trend of the development, in England, of instruments for the accurate control of the H.T.S.T. pasteurization process. As mentioned above, the subject may be somewhat beyond the strict limits of this review, but the urge towards this work has been largely applied by the bacteriologists who demand that the plant offered shall give reliable and reproducible results and shall translate the results of their researches in the laboratory into commercial practice.

Another point in plant design which is not recognized in Official Regulations, nor generally considered by plant manufacturers, is that the time required to heat the milk from a slightly lethal temperature, such as 140° F., to the pasteurizing temperature influences the result of the heat treatment. The longer the time taken the greater the effect. This point has been discussed by Ball (44). Figures quoted by Hiscox (8) indicate that in some circumstances the rate of heating may have an importance comparable with that of the holding time. This effect is taken into account in the 'Quick-time' pasteurization (27). There is no holding time as such, and the time/temperature standard refers to the time that the temperature of the milk is above 140° F., when milk is being treated at a given temperature.

Phosphatase test. The essential requirement of any pasteurization process is to produce milk free from pathogenic organisms. The time/temperature factors imposed for holder and H.T.S.T. pasteurization have been proved by laboratory experiment to offer an ample margin of safety. It would not be feasible to test all routine samples for pathogenic organisms, but the phosphatase test has been found to be a good indication of adequate heat treatment. Kay (45) discusses the test, its uses, and its applications in this and other countries. He points out that although the test will not indicate the form of heat treatment to which a sample of milk has been subjected, a positive result shows that it has not been adequately heated. Cooper, Woodman & Nicholls (46) raise the objection that unsafe milk may be distributed before the report of a positive phosphatase test reaches the dairy, some 24 hr. after the pasteurization run. They have, therefore, used a number of supplementary tests (cream rising, blood corpuscle, sedimentation, carmine and indigo suspensions) in the control of holder plants and propose extending them in the routine control of H.T.S.T. plants. Haller, Babcock & Ellis (47) found that the phosphatase test as usually carried out could not be applied to goat's milk, since milk that had been heated at 143° F. for only 5 min. would pass the test. Certain bacteria are capable of producing phosphatases and therefore of causing false-positives. But since these enzymes differ from the natural phosphatase of milk, notably in their thermostability, it should be possible to distinguish false-positives. Kaplan (48)

recommends the repasteurization and retesting of all samples suspected of faulty heat treatment. In this way he found that 50% of a group of suspected samples had given false-positives. Buck (49) isolated from the milk of four Baltimore dairies a thermophile resembling *L. thermophilus* in many respects, but differing from it in its capacity to produce a phosphatase. Its optimum temperature of growth was 52° C., but growth still occurred at 60 and 62·5° C. The enzyme was destroyed by boiling for 1 min. Andersen & Vestesen (50) have emphasized that if control is to be based on the phosphatase test then details of technique must be laid down and strictly followed. They suggest a technique based on Neave and Aschaffenburg's modification of Scharer's test. Full details of the laboratory technique followed in Great Britain have been issued by the Ministry of Health (51).

Brown & Elliker (52) found that when cream was flash-pasteurized the phosphatase test was often negative or just slightly positive immediately after the process, but that the values increased when the cream was held in cold storage. Barber & Frazier (53, 54) found a similar reversion from a negative to a positive phosphatase test in stored cream. They traced this 'reactivation' to the development of enzymes in spore-bearing bacteria which could grow at low temperatures. Repasteurization of the cream at temperatures below 76·7° C. gave a means of differentiating between the natural and the bacterial phosphatase. With the increasing demand for the manufacture of cheese from pasteurized milk, it has become desirable to adapt the phosphatase test for use with cheese. Techniques have been developed by which it is claimed that the addition of as little as 0·1% of raw milk to the pasteurized vat milk can be detected in the cheese (55, 56, 57, 58). Moulds have been shown to secrete active phosphatase, and mould-free samples only should therefore be used for the tests (58).

Other tests for the control of the pasteurization process are concerned with the destruction of non-pathogenic organisms and are therefore related to the keeping quality of the milk as it affects the dairy industry. A discussion of these will be found in the sections dealing with colony count methods and dye reduction tests.

Thermoduric organisms. Even though it may be conceded that thermoduric organisms in general are non-pathogenic and slow-growing in milk and therefore have little effect on the safety or keeping quality of pasteurized milk, they nevertheless attract a considerable amount of attention, partly because they indicate faulty methods of milk production and partly because of their academic interest. Boyce, Lythgoe, Ruggles & Lane (59) state that while strict supervision of pasteurizing plants must be maintained, the problem of improving the bacteriological quality of the milk sold in Massachusetts is largely a question of improving the quality of the milk at the farm. A case, however, is quoted in which the cause of high counts in the pasteurized milk was traced to a badly kept pasteurizing plant. The numbers of thermoduric organisms in the raw milk appear to vary with the season. Macy & Erekson (60) found that the percentage of samples that could not be pasteurized satisfactorily was greater in summer than in winter. Little (61) found a high percentage during the winter months for graded raw-milk supplies, but at no time of the year could ungraded supplies be relied upon to give low counts. Dotterrer (62) also found that thermodurics were more numerous during the colder months. McKenzie & Morrison's (63) experiments showed that thermodurics were less prevalent in the milk from individual farms than in bulk milk supplies. They suggest that this is due to the fact that a minimum of equipment is used on small farms. They sometimes obtained almost pure cultures from farm equipment, especially milking machines, suggesting that these sites provided good conditions for the enrichment of thermoduric organisms. Prouty (64) traced an outbreak of ropiness in pasteurized milk to the presence of a thermoduric *Micrococcus* in the milk of one producer. Since improved methods of production and cleaning of the utensils did not remove the trouble, and the organism resembled *M. cremoris-viscosi* isolated from the udder of a cow, it was possible that the udder was the seat of infection, though no direct evidence could be found to confirm this suggestion.

The theory that the udder is the ultimate source of the thermoduric micrococci is debatable. Abdel-Malek (65) did not find that the micrococci isolated from aseptically drawn milk resisted laboratory pasteurization at 143° F. for 30 min., or that they were identical with the strains

isolated by him from pasteurized milk. He found no evidence that the udder is the source of thermoduric organisms found in milk. Similarly, Mallmann & Bryan (66) examined milk drawn aseptically from the udders of 102 cows from three herds. Thermoduric organisms were found in only six samples. Five cows were suffering from mastitis, but no streptococci survived laboratory pasteurization. Macy & Erekson (60) rarely found udder cocci in numbers large enough to cause trouble.

Thermoduric organisms are, in general, limited to a few species of bacteria, notably micrococci, microbacteria (corynebacteria), streptococci, and spore-bearing rods. Abdel-Malek (65) has studied the distribution of these types in certified and bulk milk pasteurized in the laboratory. Streptococci were predominant among the strains isolated at 37° C., while corynebacteria predominated amongst those isolated at 30° C. There still appears to be a divergence of opinion as to whether the latter organisms should be termed corynebacteria because of their morphology, or retain the name *Microbacterium* originally given by Orla-Jensen. Abdel-Malek adheres to the name *Corynebacterium*, but Speck (67) disagrees and stresses the relationship with propionibacteria and lactobacilli. Orla-Jensen (68) points out that the microbacteria cannot be regarded as true lactic acid bacteria, but for the sake of completeness he has included them in his scheme for the interrelationships of the lactic acid bacteria. He leaves it to the future to decide whether he is right in linking them with the corynebacteria. He utters a warning against assuming that all bacteria showing diphtheroid and branched forms are related.

Comparison of holder and H.T.S.T. *processes.* The H.T.S.T. process has many advantages over the holder process, but a frequent criticism is that it is less effective in the destruction of thermoduric bacteria. This is to some extent supported by the results of the survey of pasteurizing plants made by Clegg & Lomax (9), in that the plate counts tended to be higher for H.T.S.T. than for the holder plants. Workman (69) found, during the study of more than twenty plants of four types (Cherry-Burrell, Creamery-Package, Electro-pure and York), that the numbers of thermoduric bacteria were likely to be higher with H.T.S.T. plants but not significantly so. Direct comparisons between the two processes, pasteurizing simultaneously at 162° F. for 16 sec. and at 143° F. for 33 min., were made by Fay (70). Of 262 samples 144 (55%) gave higher counts by the H.T.S.T. method and fourteen (5%) gave higher counts with the holder process. No details of the plants are given.

Millenky & Brueckner (71), using a plant of the Electro-pure type, found that a pasteurizing temperature of 165° F. was necessary to produce the same effect as holder pasteurization at 143·5–144° F. for 30 min., when the raw milk contained thermoduric organisms. Lipman, Frandsen & Lindquist (72) report that when portions of the same batch of milk were pasteurized by the Electro-pure process at 162° F. for 16 sec. and by the holding process at 143° F. for 30 min., the bacterial destruction was equal in the two processes. The general conclusion would seem to be that thermoduric bacteria are slightly less susceptible to the time/temperature conditions of the H.T.S.T. process than to those of the holder process, though it is not always clear from the reports quoted above that the samples were taken from H.T.S.T. and Holder plants running in parallel. It is probable that in some cases samples from the H.T.S.T. plant were compared with samples pasteurized in the laboratory by the holder process. In this connexion it should be emphasized that, as has been demonstrated by Ball (44), the curves showing the time-temperature combinations having a lethal effect equivalent to that of a given standard (e.g. holder pasteurization) are not necessarily of equal slope for all bacteria and enzymes. If the standards for holder and H.T.S.T. pasteurization are such that they have an equal effect on one strain of bacteria it should not be assumed that they will be equivalent for all other bacteria.

Coliform organisms. Coliform organisms are of special interest in connexion with pasteurized milk. The justice of the presumptive coliform test when used for routine control purposes is sometimes queried, mainly on the basis of evidence brought forward for the existence of heat-resistant strains which might survive the pasteurizing process.

Procter (73) and Morris (74) report the presence in raw-milk supplies of coliform organisms which survived laboratory pasteurization of this milk and suggest that these represent heat-resistant

strains. It is not stated that resistance tests were extended to include succeeding generations of these organisms, and there is therefore not enough information to decide whether their survival was due to an inherent high resistance or to a temporary resistance induced by the environmental conditions of the cells. Morris does, however, state that heat-resistant coliforms were isolated from the stall tubes and unit buckets of milking machines which had not been carefully cleaned and had a layer of dried milk on them. The organisms disappeared when the tubes and buckets were thoroughly washed and sterilized.

Crossley (75) found coliforms, after laboratory pasteurization, in only two of 484 samples of bulked tank raw milk, and in none of 498 samples direct from the individual producers. Buchbinder & Fertig (76) took 400 quart samples of milk from the holder of a plant, most of them during warm weather, and after incubating at 37° C. overnight plated 1 ml. on desoxycholate agar. Only one sample contained coliforms. Quart samples taken at the same time from the bottle-filler usually contained coliforms. Jensen (77) examined samples of milk from six dairies over a period of 2 years. All samples direct from the heater were free from coliforms. Similar results have been reported by earlier workers.

Natvig (78) found that of 159 strains of coliform organisms isolated from raw milk, bottled pasteurized milk and human faeces, none survived heating at 70–72° C. for 15 sec. Although Crossley (75) found coliform organisms in only two of 982 samples of raw milk after laboratory pasteurization, 9·1% of his strains isolated from raw milk and 34·6% isolated from pasteurized milk were resistant to heating in milk at 63° C. for 30 min. The resistant strains in the raw milk may have been derived from churns and factory equipment. If they were actively proliferating at the time of testing or of pasteurization, they would have been vulnerable to heat. Pasteurized milk from the holders contained few or no coliform organisms, and the high incidence of heat-resistant strains in samples of pasteurized milk taken at points beyond the holder was most probably due to post-pasteurization contamination from the plant. Swabbings showed that 50% of the strains from the plant were heat-resistant. Crossley suggests that the source of these strains may be, in part, the plant washing water which, being hot and containing alkaline detergents, may act as a selective agent in the accumulation of resistant cells. The process of cleaning and sterilizing the plant will, if inadequate, have a cumulative effect, and the residual coliform organisms may be expected to contain a high proportion of heat-resistant strains. These and other data collected by Crossley have thrown much light on the problem of the heat resistance of coliforms in pasteurized milk and other milk products. His figures would seem to suggest that heat-resistant strains are more numerous than has been generally believed, but since the only prolific source that has been demonstrated is imperfectly cleaned and sterilized plant and equipment, their incidence in pasteurized milk can be largely avoided by attention to details of plant cleanliness. Even heat-resistant strains are easily destroyed by heat when they are actively growing, and probably they are usually at this stage when the raw milk reaches the pasteurizing plant.

The practical importance of this question of the heat resistance in coliform organisms has encouraged investigation of a more academic nature. The rate of destruction of bacteria follows the curve of a monomolecular reaction and Katzin, Sandholzer & Strong (79, 80) suggest that the principle of Decimal Reduction Time (time required for a 10% survival of organisms under standard condition of heating) should be used for the evaluation of processes involving the thermal destruction of bacteria. They found the Decimal Reduction Time of each of sixty-six strains of coliform organisms isolated from a variety of sources was less than 2 min., usually less than 0·5 min. when heated at 61° C. (141·8° F.). They concluded that in ordinary circumstances few, if any, coliform organisms should survive pasteurization. Craige (81) showed that when twenty-four strains of the *coli-intermediate-aerogenes* group isolated from raw and pasteurized milk and ice cream were heated in milk at 62° C. for 30 min., all were able to survive if the concentration of organisms was high enough. The resistance varied from strain to strain, but in no case were there survivors in any tube containing less than 700 organisms per 1 ml. Unlike most other workers he used 48 hr. cultures on nutrient agar for the heating tests. He

pointed out that where figures are given in the published reports on heat-resistance tests on coliform organisms the initial concentration of these organisms in the test sample was often much higher than it would be in a good raw-milk supply. Nelson (82, 83) found that heat-treated bacteria (including *Bact. coli*) were more sensitive to external conditions than unheated cells, and that variation in the plating procedure might cause considerable difference in the plate counts of such organisms. The composition and *p*H of the plating medium were specially important. Thus many factors must be considered before it can be assumed that a strain of coliform organisms is truly heat-resistant.

The presumptive coliform test is no longer used in England for the legal control of pasteurized milk supplies, but it is widely used in advisory work and plant control at the dairy. There is much evidence to show that it is only in exceptional cases that coliform organisms in raw milk are not killed by pasteurization at 145° F. (62·8° C.) for 30 min. or at 162° F. (72·2° C.) for 15 sec. The presence of coliforms in pasteurized milk will therefore generally indicate inefficient pasteurization (which can be checked by the phosphatase test) or post-pasteurization contamination from the plant. Sherman (84) discusses the public health significance of coliform organisms in pasteurized milk and considers that the use of the coliform test in the detection of post-pasteurization contamination in the milk plant is one of its most valuable applications. Breed (85) drew attention to the fact that the procedure adopted by the American Public Health Association called for the use of 1 ml. of *freshly pasteurized* milk in making the tests. The standards were set up to indicate recontamination of the milk after pasteurization, and they would not be applicable to street samples of bottled milk as delivered to the consumer, i.e. after an interval during which coliform organisms may have been able to increase in numbers. The standard, which demands freedom from coliform organisms in 1 ml. rather than 10 ml. quantities of milk, allows a slight latitude for the possible presence of heat-resistant strains.

Babcock (86) points out that a milk supply should not be condemned on the basis of the presence of coliform organisms until it has been established that they are not heat-resistant strains. Care should be taken in the interpretation of the test. Craige (81) suggested that since raw-milk supplies may contain coliform organisms in sufficient number for the possible survival of some cells, it seemed impractical to insist that pasteurized milk should be entirely free from these organisms, but a limit should be set which would detect poor-quality raw milk or post-pasteurization contamination. These requirements appeared to be satisfied by the test recommended by the United States Army Medical Department (87) in which each of five tubes of brilliant green lactose bile broth are inoculated with 1 ml. of the sample of milk. The sample is considered negative if not more than two tubes show gas after incubation.

New Provisional Standards for Coliform Bacteria in pasteurized milk, which became effective on 1 June 1946, have been promulgated by the City of New York Department of Health. The ultimate objective, that all milk sold in New York City shall be free from coliform organisms, cannot be yet attained because of present limitations in operating conditions and processing technique. The standard requires that a 5 ml. sample of milk shall be tested (by plating on sodium desoxycholate agar) during the warm months, May to October (inclusive), and a 20 ml. sample during the colder months, November to April (inclusive). From May to October the appearance of ten or more colonies per 5 ml. milk or the repeated appearance of 6–9 colonies will be considered unsatisfactory. The appearance of 1–5 colonies or less will be considered satisfactory. From November to April, six or more colonies per 20 ml. (0·3 colony per 1 ml.) or the repeated appearance of 3–5 colonies will be unsatisfactory; one or two colonies per 20 ml. will be satisfactory. The data on which these standards are based were collected by Buchbinder & Fertig (76) during a survey of the performance of the forty-six pasteurizing plants of New York City. In all, 2200 samples, each a full quart bottle taken directly from the filling machine, were examined. Some of the problems studied were the seasonal variation in the proportion of coliform positive samples, the prevalence of heat-resistant coliforms, the relative merits of different media, the distribution of the numbers of coliform organisms per 1 ml in summer and in winter, and the correlation between the number of coliform organisms found in summer and winter at individual dairies.

Jansen (88) cites examples of unsuspected sources of infection of pasteurized milk due to the unavoidable use of unsatisfactory labour. In the circumstances, with an imperfectly washed plant, sterilization by passing steam through the plant was more effective than treatment with chlorine. Weber (89) has shown seasonal fluctuations in the number of positive coliform tests for pasteurized milk, although there was no corresponding variation in the plate count. The peak was in the warmest quarter of the year, i.e. July to September. He regarded the fluctuations as being due to seasonal variations in the degree of infection rather than to variations in the rate of growth. These findings have been confirmed by Buchbinder & Fertig (76).

Heat-resistance tests are generally based on holder pasteurization standards rather than on the H.T.S.T. process. This is due to lack of laboratory apparatus sufficiently delicate to avoid the great possibility of error when heating samples for merely a matter of seconds. Natvig (78) has used tubes of milk or broth adjusted to the temperature of the test (70–72° C.) by immersion in a water-bath at that temperature. A loopful of the culture to be tested was placed in the broth or milk and well mixed. At the end of the heating period (10 or 15 sec.) a loopful of the heated culture was withdrawn, placed in a tube of broth or milk at 15° C. and incubated.

Gilcreas & O'Brien (90) have devised an apparatus in which the inoculum is injected into the heating medium and the samples withdrawn by means of electrically controlled syringes.

Thermophilic organisms. Little new information concerning thermophilic organisms has been published. Imšenecki & Solnzeva (91) have studied the growth of *Bacillus diastaticus* and another thermophilic bacillus at 60° C., recording plate counts and direct counts at 4, 6, 17, 24 and 36 hr. after inoculation. The numbers of organisms increased to a maximum within 24 hr., and as rapidly declined after this point. In so far as they go their figures illustrate one of the characteristics of thermophilic bacteria—that they reproduce rapidly and as rapidly age and die. Isaacs & Gordon (92) determined the plate counts at 63° C. on samples of pasteurized milk taken at hourly intervals from four pasteurizing plants. From the figures they deduced a doubling of numbers of thermophilic organisms every 1·5 hr. that the plant was running. Samples of milk drained from the heating system at the end of the run gave high counts, indicating that this part of the plant was an 'incubator' of thermophiles. The organisms appeared to multiply as soon as pasteurization began, but they were not usually released into the milk stream within 3 hr. The instance of a thermophilic organism, capable of producing phosphatase and therefore of giving misleading phosphatase tests, cited by Buck (49), has been noted earlier in this section. Bird & Egdell (93) describe thermophilic organisms, isolated from pasteurized milk and plant swabbings, milk cultures of which were able to reduce methylene blue at 37° C. The presence of large numbers of these organisms in pasteurized milk would thus influence the methylene-blue reduction test.

(*b*) Dried-milk powder

Full-cream dried milk, and especially the spray-dried product, tends to deteriorate during storage. The chief defect is the development of an unpalatable tallowy flavour, due to oxidation of the fat. This fault is mainly of chemical origin, and the means by which it has been overcome, such as gas packing; the addition of antioxidants, etc., are not relevant to this review. Bacteria, though perhaps inert in the dry powder, may have a deleterious effect on the reconstituted product and dried-milk powder of good quality should be of high standard bacteriologically as well as chemically. In a study of the bacteriology of dried-milk powder from two plants of the Kestner type Crossley & Johnson (94) have examined the product at all stages of the process. Most of the work dealt with skim-milk powder, but whole-milk powder and dried whey were also studied. The plate count of 671 powder samples varied from 200 to 19,500,000 per g. The flora (as determined by plating on standard milk agar at 37° C.) consisted essentially of streptococci and micrococci of comparatively few species. Aerobic spore-forming bacteria and *Achromobacter* spp. were found regularly but not in significantly large numbers, and anaerobic spore-forming bacteria were found occasionally. Coliform organisms were rarely isolated from 1 ml. of the reconstituted milk though they were sometimes found in 20 ml. samples and false-positives were

common. 48·5% of the strains isolated were heat-resistant. A great reduction of organisms, varying with the quality of the raw milk, occurred during the pre-heating process (at temperatures ranging from 159 to 164° F. for 20 sec.). The evaporators, which were of a Kestner triple effect climbing film, were potential sources of reinfection, since they are difficult to clean and sterilize. The numbers of bacteria in the milk frequently increased as the milk passed over these evaporators, but it was possible to obtain concentrated milk of low bacterial count and free from coliform organisms by careful plant cleaning. The benefit of a clean and sterile plant was also shown by a later experiment (Mattick, Hiscox, Crossley, Lea, Findlay, Smith, Thompson, Kon & Egdell (95)) in which the influence of various factors on the keeping quality of whole milk dried by the Kestner spray process was studied. The standard of cleanliness was steadily raised during the control stages of the experiment, and once the maximum was reached little recontamination of the milk occurred as it was passing through the evaporators, and the concentrated milk was free from coliform organisms. The use of a selected milk supply, combined with a clean plant, gave a dried-milk powder with a plate count at 37° C. of only a few hundreds per g. There was no good evidence that clarification of the raw milk affected the plate count of the powder. The most striking effect was attained when the pre-heating temperature was raised from 165 to 190° F. The plate counts were lower (this was more clearly shown when the plates were incubated at 30° C.), and powder of a high bacteriological standard and good keeping quality was obtained. By using raw milk of low bacterial count and pre-heating at 190° F. a powder was obtained which had a storage life (in air) of 2½ years, a considerable increase on the 5–6 months' life of the powder from unselected milk pre-heated at 165° F. No doubt the more effective destruction of bacteria and enzymes aided in attaining this result, but the greatest effect was due to the production in the milk of antioxidant-active sulphhydryl compounds. The solubility of the powder was not appreciably reduced, but it acquired a slightly 'cooked' flavour. Similar results obtained by raising the pre-heating temperature in a Krause plant are described by Findlay, Higginbottom, Smith & Lea (96). In examining some 400 samples of dried milk products from a group of factories in Great Britain, Higginbottom (97) found marked differences between roller and spray-dried products. The mean plate counts were much higher and positive presumptive coliform tests more numerous for the spray-dried products. Some plants gave consistently low counts, others gave higher and variable counts. The flora of the roller-dried product consisted chiefly of micrococci, sarcinae and spore-forming bacilli, the spray-dried almost entirely of *Str. thermophilus* and *Str. faecalis*. Higginbottom (98) also showed that the decrease in the plate count at 37° C. of milk and whey powders during storage may vary with the type of powder. More than 40% of the milk powders stored for 3 months showed a decrease of more than 50%, but 30–40% showed less than 20% decrease. Some showed little alteration during 6 months or longer. Decreases were slightly less in roller- than in spray-dried powders.

The method of reconstitution of dried-milk powder and the temperature of incubation of the plates may have a considerable influence on the plate count. Thus, Hiscox (99) found that samples of spray-dried milk powder gave higher plate counts when reconstituted at 50° C. than when reconstituted at room temperature. Warming to 50° C. after reconstitution at room temperature did not increase the count. Plates incubated at 30° C. gave higher counts than those incubated at 37° C., and 35° C. appeared to be a critical temperature. The difference in count appeared to be due to the presence of microbacteria (corynebacteria) which grow only feebly or not at all at 37° C. and therefore have not been recorded in earlier studies of the flora of dried-milk powder. These organisms were predominant in the powders made from milk pre-heated at 165° F., but they were usually outnumbered by the micrococci and the spore bearers in the powder from milk pre-heated at 190° F. This effect of the temperature of reconstitution and of incubation has been confirmed by Higginbottom (100) for samples of spray-dried full-cream milk powder from eight plants in Great Britain, and for samples of full-cream roller-dried milk powder.

(*c*) Ice cream

In the eighth edition of the American *Standard Methods for the Examination of Dairy Products* procedures for the examination of ice-cream ingredients are included. For control purposes and for the proper interpretation of the results of tests on the freshly frozen product, it is obvious that a knowledge of the source of the bacteria present—the numbers contributed by the individual ingredients which are included in the mix, especially the numbers likely to survive under the conditions of heat treatment used, the numbers added by those materials which are incorporated in the mix after pasteurization, and the numbers derived from the surface of equipment—would be of the greatest value. The importance as sources of contamination of such ingredients as fresh fruits, nuts, flavourings and colour solutions which are usually added to the mix after pasteurization has again been shown by Prucha (101) and Tracy (102). Colony counts of samples of these materials as used at ice-cream plants were found to vary from a few hundred to several millions per g., and many contained coliform organisms. Boyles (103) obtained counts ranging from 10 per ml. to 26 millions with ninety-eight samples of dyes used for colouring ice cream; twenty-six samples had counts greater than 400,000 and seventeen contained coliform organisms. Tracy (102) was able to sterilize nut meats satisfactorily by boiling in 50% sucrose or in butter; contamination from colour solutions was prevented by preparing fresh solutions weekly in water at 180° F. and storing in sterile bottles at 40° F.

Times and temperatures for the pasteurization of ice-cream mix vary widely, and adequate information on the subject is lacking. The Ministry of Food require that mix containing dried egg, which may be a source of *Salmonella*, should within 1 hr. of its preparation be pasteurized at a temperature of not less than 165° F. for 30 min. The Frozen Desserts Ordinance proposed by the United States Public Health Service (104) advocates pasteurization at a temperature of not less than 155° F. for 30 min. A temperature higher than that used for milk is usually employed, partly because of improvements in certain physical properties of the mix and partly in the belief that bacteria are more resistant in ice-cream mix than in milk. Paley & Isaacs (105), using a 24 hr. old culture of a freshly isolated strain of *Bact. coli* with an inoculum of approximately 1 million per ml. in milk at 143° F., found that the organisms were killed in 20 min., whereas in an ice-cream mix a few cells survived this treatment for 35 min. Addition of separate ingredients to milk showed that butter, flavour, colour, sugar and gelatin were without effect on the survival of the organisms, but sodium alginate and locust-bean gum exerted a protective action. The survival of coliform organisms in mix pasteurized at 145° F. for 30 min. has also been shown by Nelson (106), but at 150° F. they were killed in 10 min.; to destroy phosphatase, treatment at 150° F. for 30 min. was found necessary. He regards the presence of coliform organisms in phosphatase negative samples as evidence of post-pasteurization contamination. Crossley (75) found a high incidence of infection with coliform organisms in samples of ice-cream mix taken before heat treatment, and at various stages in processing subsequently: coliform organisms were absent from samples taken immediately following heat treatment. He ascribed the presence of coliform organisms in ice cream to contamination following heat treatment. Brannon (107) believed that pasteurization at 150–160° F. for 30 min. would destroy all pathogenic organisms. Dowd & Anderson (108) obtained negative phosphatase results when mix was pasteurized at 180° F. for 19 sec. using an Electro-pure unit, and the results were considered equal to those obtained by pasteurization at 160° F. for 30 min. In experiments on vacreation (109) it was found that the high temperature used (195° F.) led to an improvement in the bacteriological results and keeping quality of ice cream and was without detrimental effect on its flavour.

In an attempt to explain occasional high counts in samples of ice cream even when great care was exercised in sterilization of plant, Nelson (110) studied the changes in bacterial count and coliform content of mix stored at different temperatures while awaiting freezing. At temperatures below 42° F. there was no appreciable increase in count during a 4-day storage period; when temperatures approached 49° F. there was usually a considerable increase in 4 days, and in the region of 60° F. counts were excessive after 2 days' storage; considerable multiplication of

coliform organisms occurred when storage temperatures exceeded 49° F. He deprecated the practice of making or purchasing mix and storing at moderate refrigeration and advocated storage of frozen ice cream rather than the unfrozen mix. The importance of immediate freezing of mix or its storage at a low enough temperature is emphasized by a recent outbreak of staphylococcal food poisoning (111) in which it was confirmed that the mix had been contaminated by one of the persons who had participated in its preparation. The mix had been allowed to cool down by itself and had been stored at room temperature overnight before its dispatch for freezing. Ice cream made from mix which had been allowed to stand at room temperature for 11 hr. was also suspected to be the cause of another outbreak of food poisoning (112).

At one time it was thought that the increase in count during freezing was entirely the result of disruption of clumps, but the importance of adequate cleaning and sterilization of the freezer itself is now recognized. Fabian, Cook & Nielsen (113) compared different methods of sterilizing ice-cream freezers and came to the conclusion that for efficient sterilization by any method, a thorough preliminary cleaning was of the greatest importance. Of the methods of sterilization used, steaming until the condensate reached 190° F. was found to be the most effective; chlorination with 100 p.p.m. of available chlorine was less satisfactory and, owing to rapid cooling, treatment with water at 200° F. was the least satisfactory.

A test for the presence of coliform organisms has been used by the majority of workers for the examination of ice cream. As already stated there is some doubt as to the temperature and time of pasteurization for their destruction in ice-cream mix, but even allowing for the possible survival of a few cells their presence in more than minimal numbers in the frozen product is evidence either of contamination during handling after pasteurization or of proliferation due to storage of the unfrozen mix at too high a temperature. Methods for their estimation in ice cream have been studied by Nelson & Foltz (114) using brilliant green lactose peptone bile, desoxycholate agar and violet-red bile agar. The liquid medium gave the highest number of positives (confirmed) and desoxycholate agar the lowest; most of the discrepancies occurred with samples containing small numbers of coliform organisms. Fournelle & Macy (115) similarly found little difference between counts on violet-red bile agar and in brilliant green (1:30,000) lactose peptone bile, although with the latter medium it was possible to detect a smaller number by inoculating 10 or 1 ml. than when using the agar medium. In ninety-nine samples of ice cream with coliform counts per ml. ranging from 0 to 101,000, the coliform flora consisted mainly of *Bact. cloaceae*, *Bact. aerogenes* and *Bact. freundii*; *Bact. coli* was only occasionally found. Crossley (75) found no *Bact. cloaceae* types among fifty-six cultures tested; 39·3% were *aerogenes*, 35·7 *coli*, and the remainder were Intermediate and Irregular types.

The possibility of using other methods for testing ice cream has been explored. Bryan, Scheid & Neuhauser (116), using the new American standard medium, found that counts by the Frost little-plate method and by the usual plating method were approximately the same. Long & Hammer (117), applying a modification of the Burri smear technique to the examination of ice cream, obtained higher counts than by the plate method, and considered its main value to be in the examination of the distribution of organisms in the product rather than for an exact measure of the total count. Nelson (118) found that either the time to reduce to the pink stage with resazurin or the stage of reduction after 3 hr. incubation at 37° C. had possibilities as a method of segregating the majority of ice-cream samples with high colony counts from those with low counts. A study of the results he obtained shows, however, that for samples with counts between 100,000 and 200,000—the maximum count for grading purposes usually suggested—the time taken to reduce to pink ranged from 2½ to more than 9 hr., and the 3 hr. test showed results ranging from no change in colour to complete reduction.

There is a growing demand for bacteriological standards for ice cream, and according to Fabian (119) colony count standards ranging from 75,000 to 500,000 per g. have already been imposed in twelve States in America. He favours a standard of not more than 100,000 per g. for all frozen desserts. The United States Public Health Service in its Frozen Desserts Ordinance (104) advocates a standard of 50,000 for ice cream produced at grade A plants and 100,000 for that

produced at grade B plants. Blair(120) in New Zealand and Compton & Youssef(121) in Egypt are agreed that regular sampling and constant supervision of manufacturing methods with imposition of a standard would lead to an improvement in quality. Blair(120) found that the product leaving the factory from which his samples were taken generally had counts below 50,000 with coliform organisms rarely present, although counts increased at the consumer stage due to unhygienic selling conditions. Compton & Youssef(121) consider a plate count standard (nutrient agar) of 100,000 too lenient.

V. DETERGENTS AND DISINFECTANTS

A memorandum(1) outlines in simple terms the function, composition, preparation and use of detergents. Information of a similar character has been given by McDowall(2), Parker(3) and Rahn(4).

Methods for the appraisement of detergents continue to receive attention. Liddiard & Harwood(5) have devised an electrical method for measuring the ease of rinsing of detergents from glass surfaces. A piece of glass $5 \times 2 \times 0{\cdot}5$ cm. clamped between two electrodes and connected to a milliammeter under a potential difference of 7 V. is lowered into the detergent solution, and the change in current passing after immersion for 1 sec. in successive lots of rinse water indicates the amount of alkali remaining in the film. Five per cent solutions of Na_2CO_3 and NaOH required respectively four and nine rinses; addition of 1% commercial sodium hexametaphosphate to NaOH reduced the number of rinses required to five. Davis, Ward & Liddiard(6) have described an instrument, which is a modification of the wheatstone bridge, for measuring the strength of detergents in bottle-washing machines. Tests to evaluate the cleansing efficiency of detergents have been elaborated by Gilcreas & O'Brien(7) and Hughes & Bernstein(8). Thoroughly cleaned microscope slides or plate-glass squares of uniform thickness, after treatment with a synthetic soiling mixture, were mechanically washed under controlled conditions in detergent solutions in water, adjusted to a standard degree of hardness, and the residual film measured. Gilcreas & O'Brien used a photoelectric colorimeter to measure the residual film, its intensity being expressed in terms of the difference in light transmission between control clean slides and washed soiled slides. To obtain larger differences in light transmission between clean slides and those having a residual film, slides were treated with activated carbon before taking readings. Hughes & Bernstein measured the proportion of incident light scattered by the residual film. This they accomplished by an arrangement whereby light from a parallel beam of constant intensity is prevented from reaching the active surface of a photoelectric cell, but a proportion of the light which is scattered when the glass squares are introduced into the light beam is allowed to do so, and this is recorded in terms of galvanometer deflections. Laboratory methods simulating practical conditions, of evaluating the germicidal efficiency of detergents have been elaborated by Mewes(9). Dirty bottles were washed under controlled conditions and the number of colonies per sq.cm. developing in an agar medium spread over the inner surface used to assess the comparative efficiency of different detergents. Small pieces of soiled tinplate washed under controlled conditions were used to measure the suitability of detergents for can washing; the number of organisms surviving was assessed either by a rinse method or by imprints on the surface of agar plates.

Tests by Hobbs & Wilson(10) of the destruction of *Bact. coli* and a sporing organism by NaOH, showed that the rate of disinfection was not noticeably affected by the presence of 0·1% milk, a result which is at variance with that found by Mattick & Sharpe(11), in which the presence of milk led to a considerable lowering of the k value. To meet the difficulty arising out of variations in the reaction velocity which may occur during the progress of disinfection Hobbs & Wilson(10) suggest that k should be calculated for the middle stage of the reaction, and Withell(12) suggests that the time taken to destroy 50% of the organisms should be selected as a comparative measure of bactericidal efficiency. Results based on the destruction of 25% of *B. subtilis* spores were used by Hobbs & Wilson(13) in the compilation of a table of combinations of concentration of NaOH.

time of contact and temperature suitable for cleaning milk bottles. The concentration of NaOH given is greater than that found effective in practice in bottle-washing machines by Mattick & Hoy(14). It has been emphasized(1) that the results of laboratory tests, especially when using highly resistant spores, may bear little relationship to the germicidal efficiency of detergents in practice, when the majority of the organisms to be destroyed are in the vegetative state, for as shown by Hobbs & Wilson(13) the k value of a 0·5% solution of NaOH is about 3 million times greater for *Bact. coli* than for *B. subtilis* spores.

Ames & Smith(15), in experiments on the destruction of *Bact. coli* in water in the presence of 0·25% nitrogen, found that even in the range 10–40° C. the rate of disinfection by chlorine was markedly affected by temperature. Weber & Levine(16), using the time to kill 99% *B. metiens* spores, found that doubling the concentration of chlorine reduced the killing time by approximately 50%; a drop of 10° C. in temperature led to a twofold increase in killing time.

To obviate recontamination of bottles which, in properly operated machines, are usually satisfactorily sterilized in the detergent section, Hobbs & Wilson(13) advocate a hot rinse at not less than 120° F. for the removal of detergent, the bottles thereafter being cooled by external rinses. Crowther & Berry(17) advise the use of chlorine maintained at a strength of 50 p.p.m. in the recirculated warm rinse, although Hobbs & Wilson(13) have emphasized the difficulty of controlling the dosage when the organic matter content of the water varies from hour to hour as in bottle-washing machines.

The destruction of mastitis streptococci gaining access to water used for washing udders by 200 p.p.m. of available chlorine has been confirmed by Bryan, Darby, Mallmann & Corbett(18) but at these concentrations solutions rapidly lost their effectiveness owing to the presence of organic matter. Soap solutions and sulphonated alcohols were ineffective with many strains of streptococci, *Staph. aureus* and *Bact. coli*. Byers & Ewalt(19) found that dipping teat cups and washing udders in water containing 150 p.p.m. available chlorine reduced the colony count of fresh milk by 34%; after washing seven cows the chlorine content dropped to 50 p.p.m.

In England and Wales the previous ban upon the use of hypochlorites for the sterilization of dairy utensils other than milking machines was relaxed during the war emergency. Only sodium hypochlorite solutions of approved strengths and containing not less than 0·7% sodium chlorate to enable their detection if deliberately added to milk are permitted. It has been found that small additions of sodium hypochlorite to milk oxidize ascorbic acid almost completely(20). Barkworth(21) has shown that the methylene-blue reduction time of milk samples may be increased by the addition of hypochlorite to milk, an increase with different samples of 0–5 hr. and 0–3 hr. when 180 and 10 p.p.m. available chlorine respectively were added. Hauser & King(22), using the colony count method, found that the addition of 10 p.p.m. available chlorine was apparently without effect on the rate of bacterial growth in raw and pasteurized milk kept at 50° F.; growth rate was slightly retarded with 50 p.p.m. and more so with 100 p.p.m. In the presence of 10 p.p.m. the time taken for raw milk at 50° F. to sour was increased from 4 to 7 days.

Anticipating the more general use of hypochlorites and realizing the importance of their correct application, details of methods suitable for farm and dairy utensils generally have been given by Mattick, Hoy & Neave(23). They recommend a strength of 250–300 p.p.m. available chlorine, and stress the importance of their application to thoroughly cleaned surfaces and a sufficient contact time. Brushing is recommended, or in the case of large surfaces spraying, with a solution of the hypochlorite and 0·25% of soda-ash or other suitable detergent at a temperature not less than 110° F. after a preliminary thorough rinse in cold or warm water to remove milky residues. The water used for the final rinse should contain 10–50 p.p.m. of available chlorine when there is doubt as to its purity. Once weekly sterilization by steam or boiling water, especially during warm weather, is advocated. In experiments using these methods but with cold hypochlorite-soda solutions, on two Accredited farms, one milking by machine and the other by hand, Rowlands(24) obtained good results in the early part of the year when the weather was cool, but difficulty was experienced in meeting the Accredited requirements with the advent of warm weather. By boiling the utensils once a week no further difficulty was experienced except during

an extremely hot week in August. Phillips & Thomas(25) obtained slightly better results by steaming milking-machine units on a jet for 3 min. than by soaking units between milkings in brine-hypochlorite containing 200 p.p.m. of available chlorine, but both methods were superior to soaking the units in 0·5% sodium metasilicate (aluminium alloy teat cups). In tests covering a full year on two farms the bacteriological results of milk tests were at all times excellent when units, after washing in water and hot alkali, were soaked between milkings in a solution containing 200 p.p.m. of available chlorine: where the units were merely rinsed with hypochlorite solution of the same strength, the geometric mean count of milk samples during July and August exceeded 500,000 per ml. Johns(26) continues to advocate treatment of milking-machine rubbers with cold 0·5% NaOH and claims that 5 min. treatment with this strength solution is sufficient to maintain them in a sanitary condition. In a study of methods of cleaning milking machines extending over a period of 2 years(27, 28, 29) it was found that the colony counts of milk samples were lower when units were filled with 0·5% NaOH between milkings and dismantled once weekly than when they were dismantled daily; the former method effected a weekly saving of 30–60 min. per 2-unit machine. Colony counts were, surprisingly, lower for the quarter September–November than for the December–February quarter.

In New Zealand, machines of the releaser type are in common use. To meet emergency fuel shortages which may render impossible the official method whereby each unit is flushed with 4 gal. of boiling NaOH solution followed by 1 gal. of boiling water, the soda and boiling water rinse may be reduced to 1 and 0·5 gal. respectively if the machines are first thoroughly flushed with cold water(30). Similar methods to those in use in New Zealand are advocated in Australia(31). Moir(32) has stressed the importance of using NaOH where the volume of boiling water is inadequate. A preliminary report(33) of trials of the immersion method using 0·5% NaOH indicated that while this treatment may be suitable for the liners and milk tubes other methods are necessary for the overhead pipe line and releaser; their treatment with warm NaOH and a rinse with hypochlorite solution yielded promising results. Sherman(34) found that a rinse with 100 p.p.m. available chlorine just before milking gave better results than immersion of the rubber parts in chlorine solution between milkings.

Conservation of rubber assumed added importance during the war years. Moir(35) has observed that discarded teat-cup liners can frequently be made fit for use again by prolonged boiling in strong NaOH solution. Liners were found to contain an average of 10% by weight of fat; in some parts the fat content reached 30%. Kristensen(36) has found that rubbers regularly treated by flushing with boiling NaOH solution are kept freer of fat than when Na_2CO_3 is used.

Churns which are inefficiently washed and sterilized at dairies may contain innumerable bacteria by the time they arrive at farms where, in consequence, their adequate sterilization may prove difficult if steam is not available. The introduction of the National Milk Testing and Advisory Scheme has focused attention on the problem and for the guidance of dairies the Ministry of Agriculture and Fisheries has issued memoranda dealing with methods for hand washing(37) and the operation of mechanical churn washers(38). Where hand washing is practised, steaming of both churns and lids for 2 min. is recommended, in the interest of securing churns which finish dry and bacteriologically satisfactory. For efficient operation, maintenance of an adequate temperature in the various sections of mechanical washers is considered essential, and a final drying with hot air should ensure dry churns. It is pointed out that excellent results may be obtained without using detergents. Thomas, Dixon & Jones(39) found that at many dairies in Wales churns after hand washing were steamed for only 10–30 sec., and that the bacteriological results of rinses were in consequence unsatisfactory. The importance of steaming lids as well as churns has been confirmed by Jamieson & Chen(40). Results obtained by Bowyer, Fischer, Cooper-Willis, Davis & Michaelis(41) when churns and lids were filled with a solution containing 250 p.p.m. available chlorine and emptied after 130 sec. were not as good as steaming for 30 sec., when churns were tested within ½ hr. of washing, but at 24 hr. old they were slightly better, the traces of chlorine left in the churns having a retarding effect on bacterial multiplication. In their experiments, odd churns were encountered which proved more difficult to sterilize. The same

phenomenon has also been observed by others, and Rowlands(42) has shown that it is in no way connected with the bacteriological quality of the raw milk contained in the churns before washing. Neave(43) has shown that the condition of utensils has an important bearing on the efficiency of sterilization by hypochlorites. Of churns which were free from rust, 92% gave satisfactory bacteriological results when, following washing, they were treated with a solution containing 200 p.p.m. of available chlorine, whereas of those having rusty areas, only 75% were satisfactory; when similar treatment was given to churns which had contained sour milk, the percentages satisfactory were 44 and 14 respectively.

Neave(44) obtained excellent results for churns washed by a hand-operated rotary mechanical washer which was thought to be worn out. When 2–3 churns per min. were washed, and care taken to maintain the detergent at a strength equivalent to 0·5% NaOH and to ensure a high enough temperature in the various sections, the results even after 28 hr. at atmospheric temperature were satisfactory. Crowther & Berry(17) advocate the use of hypochlorite at a strength of 300 p.p.m. of available chlorine in the final hot rinse section of mechanical washers which are otherwise giving unsatisfactory results. No data are given as to the possible corrosive effect of such solutions, although it is obvious that not only churns, but also pumps and tanks, may be affected by such concentrations.

The use of acidified steam for the final treatment of churns has received considerable attention in America. According to Parker(45, 46), when alkalis are used in detergent tanks of churn-washing machines, accumulation of proteins in the solution leads to deposition of a film on the surface of churns, which, in spite of maintaining the temperature at 170° F. or higher and controlling the alkalinity at 0·25% Na_2O, leads to increasing difficulty in obtaining sterile churns as the run progresses. The flora of such churns was found to consist mainly of Gram-negative organisms, proteolytic and oxidizing types of the genera *Pseudomonas* and *Achromobacter* predominating. Final treatment of the churns with steam charged with gluconic acid in sufficient concentration to reduce the *p*H of the churn surface to between 6·0 and 6·5 gave lower counts and suppressed the Gram-negative flora. Even better results were obtained when the alkali was replaced by a proprietary mixture, consisting of organic acids, wetting agents and corrosion-inhibitor. Results of a comparison in mechanical churn washers of acid with alkali detergents are also given by Schwarzkopf(47, 48) and Scales(49, 50). Both agree that acid detergents give better results, are less costly and less corrosive than alkali detergents maintained at a strength equivalent to approximately 0·05% NaOH.

Many large pieces of equipment used for milk handling are not easily sterilized by steam, and possible alternative methods for their sanitation have been outlined(51). Neave & Hoy(52) were able to sterilize well-cleaned large stainless steel milk tanks with smooth surfaces by spraying with a cold solution of 200 p.p.m. of available chlorine and 0·25% soda-ash at the rate of 1 gal. per 40 sq.ft. of surface. Bearings and rough surfaces proved difficult to sterilize by this method. In the United States(53) spraying for not less than 5 min. with a solution containing not less than 250 p.p.m. of available chlorine is recommended, and the need for adequate cleaning and sterilization of road and rail tanks both at the emptying and filling dairies is emphasized.

Surface-active agents are being incorporated in some proprietary detergents used for the cleaning of dairy equipment, but data of their effectiveness in practice are very limited. The value of quaternary ammonium compounds for the sterilization of dairy equipment has received attention by some American workers. Rahn(4) has shown that, like chlorine, there is some loss of efficiency in the presence of organic matter, and that their bactericidal efficiency is much reduced if applied to equipment which is not thoroughly washed. Krog and Marshall(54) found that the use of alkyldimethylbenzyl ammonium chlorides at a concentration of 1:6000 (170 p.p.m.) for the final rinse of a large pasteurizing plant reduced by 60–98% the number of bacteria recoverable by a swab method. Using the same compound at a concentration of approximately 800 p.p.m. for spraying washed milk churns before use at farms Jamieson & Chen(55) obtained a reduction of 96–99% in the number of recoverable bacteria by a swab method: a similar reduction was secured by spraying churns with hypochlorite solution containing approximately

183 p.p.m. available chlorine. Tests by Frayer(56), using an inoculum of approximately 5 million viable cells per ml. of 20–24 hr. old broth cultures of *Bact. coli*, *Bact. aerogenes* and *B. subtilis* in solutions of this germicide in water, showed that a strength of 400 p.p.m. there were no survivors after a 15 sec. time of contact with *Bact. aerogenes* and *B. subtilis* and after 30 sec. with *Bact. coli*; using solutions containing 200 p.p.m. there were no survivors after 1 min. with *Bact. aerogenes*, and after 3 min. with *Bact. coli* and *B. subtilis*. Valko & Du Bois(57) have shown that the antibacterial action of surface-active cations can be reversed under certain conditions by anion active agents. Using sodium dodecyl sulphate as antagonistic agent they found that it was possible to reverse the action of a 1:3000 dilution (330 p.p.m.) of a mixture of alkyldimethylbenzyl ammonium chlorides after action on a 22–26 hr. old culture of *Staph. aureus* for 10 min. but not after 30 min. This factor has apparently not been taken into account by workers in the field of dairying although Jamieson & Chen claimed that in their work, immediate transfer of swabs into 9 ml. of sterile skim milk minimized the carry-over effect of the germicide. Lawrence(58) has reviewed the use of quaternary ammonium compounds in dairying.

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I. MILK CONTROL

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